A. M. A.
ARCHIVES OF
PATHOLOGY

MARCH 1953

VOLUME 55 NUMBER 3

Published Monthly by

AMERICAN MEDICAL ASSOCIATION

535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

TABLE OF CONTENTS FIRST PAGE

SMALL-GROUP STUDY IN A NORMALLY LIT ROOM

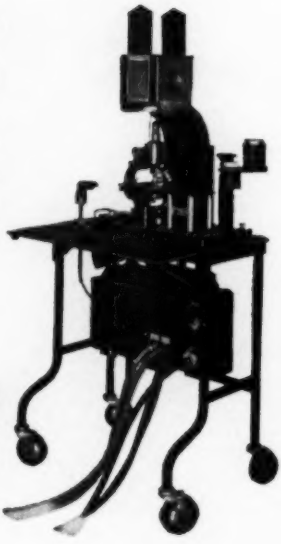
The image is cast upon a screen in the dark-chamber turret at table height. Each observer (up to 10) has a separate light-excluding viewing hood. The pathologist here is discussing a slide with the surgeon; the unused hoods are easily shuttered off.

LARGE-GROUP EXHIBITION IN THE DARKENED AUDITORIUM

So intense is the Scopicon light source that images up to ten feet across can be projected. Through a system of auxiliary lenses the operator can follow a biological specimen through a progressive series of ever-closer localizations, up to ultimate demonstration under oil-immersed objectives.

2 or 10 or 1000

Quickly adaptable to viewing on any scale, the mobile Scopicon couples convenience with efficiency. Its high-pressure mercury arc is the brightest light source ever employed for microprojection. The focal spot is only 1mm square (approx.). Its pinpoint character permits flickerless projection of sharply detailed images up to ten feet across *even under oil-immersed microscope objectives.* Let us send you the brochure describing this remarkable instrument.



Scopicon

micro-projection equipment

SCOPICON, Inc. 215 E. 149 ST. NEW YORK 51

CONTENTS

Original Articles

Case Reports

General Reviews

Laboratory Methods and Technical Notes

Regular Departments

THE A. M. A. ARCHIVES OF PATHOLOGY is published by the American Medical Association as a medium to advance pathology in the United States and to promote research and observation in this field.

Communications regarding subscriptions, reprints, etc., should be addressed, A. M. A. ARCHIVES OF PATHOLOGY, American Medical Association, 535 North Dearborn Street, Chicago 10.

Manuscripts for publication, books for review, and correspondence relating to contributions should be sent to Dr. Granville A. Bennett, Chief Editor, 1853 West Polk Street, Chicago 12, or to any other member of the Editorial Board.

Articles are accepted for publication on condition that they are contributed solely to the A. M. A. ARCHIVES OF PATHOLOGY. Manuscripts must be typewritten, preferably double spaced, and the original copy should be submitted. Zinc etchings and halftones will be supplied by the Association when the original illustrations warrant reproduction and when their number is not considered excessive.

Footnotes and bibliographies (the latter are used only in exhaustive reviews of the literature) should conform to the style of the *Quarterly Cumulative Index Medicus* and include, in the order given: name of author, title of article and name of periodical, with volume, page and year.

The A. M. A. ARCHIVES OF PATHOLOGY is published monthly. The annual subscription price (for two volumes) is as follows: domestic, $8.00; Canadian, $8.40; foreign, $9.00, including postage. Current single copies, $1.00, postpaid, except special issues.

Checks, money orders and drafts should be made payable to the American Medical Association.

Simple Precise

Control...

of Prothrombin Time with

Solu-Plastin®

(THROMBOPLASTIN SOLUTION - SCHIEFFELIN)

SOLU-PLASTIN gives you these extras . . .

SIMPLE—Solu-Plastin is simple and easy to use. No extra work of preparation is required because it is supplied as a stable solution.

PRECISE—Prothrombin times are accurate, consistent and reproducible.

ECONOMICAL—Only the actual amount needed is used. No waste.

STANDARDIZED—**Every rigidly controlled lot** is standardized against normal and dicumarolized human plasma.

Supplied: 10 cc. bottles (with the same amount of 0.0125M Calcium Chloride Solution)

Schieffelin — Quality Integrity Service

Write for literature and ***LARGE DIRECTION CARDS.***

Schieffelin & Co. / ***since 1794***
pharmaceutical and research laboratories
18 Cooper Square, New York 3, N. Y.

MODEL PR-1

Improved Model

International Refrigerated Centrifuge

The New International Model PR-1 Portable Refrigerated Centrifuge embodies the many time-proven features found in previous models and, in addition, incorporates important engineering improvements. The result is superior performance in the laboratory without increase in cost.

A new type of temperature controller enables the user to pre-select the operating temperature within the Centrifuge chamber. Temperature is controlled within ±1° C. and indicated on a calibrated dial.

An autotransformer speed controller is provided with alternating current models, eliminating heating associated with the rheostat type of controller, and providing uniform, stepless speed control throughout the entire range.

A new hinged panel provides easy access to all instruments and electric controls, thus facilitating maintenance.

A hermetically sealed unit is substituted for the open type compressor heretofore used. This eliminates troublesome vibration caused by the action of the compressor and minimizes the possibility of gas leakage.

Interchangeable accessories are available in wider variety, enhancing the usefulness of the Centrifuge.

Send today for new descriptive Bulletin RC-1952 containing complete details and current prices.

INTERNATIONAL EQUIPMENT COMPANY
1284 SOLDIERS FIELD ROAD, BOSTON 35, MASS.

for better Autopsy

Hydra-Sana-Rest

Completely Adjustable Autopsy Support

Hydra-Sana-Rest fits any autopsy table. *It provides cleaner dissection because it holds the body three inches above the table, permitting a free flow of water under the body.* Adjusts to any body size, giving firm support at head, shoulders, arms, buttocks and feet. Head assembly raises, lowers and pivots to facilitate cranial autopsy. Exposes more body area, affording more precise incisions.

No. 62755 • Hydra-Sana-Rest Autopsy Support . . . $157.50

Pan Type Balance

Extremely accurate weighing without eyestrain. Simply slide beam weight to coarse position, then turn dial for fine adjustment. This precision balance has simple zeroing device and positive beam stop. Finished in durable white enamel with removable chromium finish pan.

No. 62708A • 13" x 8½" x 5¼"
6K Capacity, 1 g. Sensitivity **$42.50**

No. 62708B • 15" x 10½" x 5¾"
15K Capacity, 3 g. Sensitivity **$49.50**

Autopsy Knives

Hollow ground blades, made of finest surgical steel. Firm grip aluminum handles fit hand like a scalpel. Honed and stropped to extremely keen edge—ready for use when received.

No. 62512 • Autopsy Knives, sold individually
A • 200 x 15 mm (blade size) **$12.00**
B • 150 x 15 mm (blade size) **9.00**
C • 90 x 15 mm (blade size) **5.50**

No. 62510 • Autopsy Knives, set of three in hinged wooden case. Set **$26.00**

the first name in hospital supplies

American Hospital Supply corporation

GENERAL OFFICES • EVANSTON, ILLINOIS

PROPPER
Select Quality

Micro Cover Glasses

The Cover Glass with the P-H-P Factor

SQUARES, RECTANGLES AND CIRCLES

The finest quality cover glasses provide maximum optical clarity with no distortion. To attain this select quality, cover glasses *must have all three* of these characteristics:

1. *Planeness—absolute flatness of surfaces*
2. *Homogeneity—uniformity of quality and composition*
3. *Parallelism—absolute uniformity of thickness*

Individually, these factors fail to give top quality. Without all three, it is impossible to secure freedom from optical distortion.

Unlike window glass, which is drawn in strips, Propper Select Quality Cover Glasses are precision-blown by craftsmen of long experience to achieve the true P-H-P Factor.

Propper Select Cover Glasses are made by an exclusive process . . . different from that of any manufacturer in the world. It is this unique method that makes possible a superior glass . . . non-corrosive, non-fogging, extremely hard and yet not brittle and offering the full P-H-P Factor.

Planeness—Homogeneity—Parallelism

When placing your next cover glass order, remember to consider all three parts of the P-H-P Factor, and specify America's Leading Cover Glass—Propper Select Quality.

PROPPER MANUFACTURING COMPANY • INC •
10-34 44TH DRIVE, LONG ISLAND CITY 1, N. Y.

A. M. A.
ARCHIVES OF PATHOLOGY

VOLUME 55 MARCH 1953 NUMBER 3

HISTOGENESIS OF BREAST TUMORS IN RATS RECEIVING 2-ACETYLAMINOFLUORENE

RODERICK C. ROSS, M.D., M.Sc.
ROBERT F. SCARF, M.D.
AND
STANLEY C. SKORYNA, M.D., M.Sc.
MONTREAL, CANADA

ADMINISTRATION of 2-acetylaminofluorene (2-AAF) to rats, in our experience, most frequently produced tumors in the auditory compound sebaceous gland.[1] In female rats the second most common site for the development of tumors was the mammary gland. Since some aspects of these growths corresponded closely to human breast tumors, a comparative study was undertaken.

The incidence of breast tumors produced by 2-acetylaminofluorene has been variously reported for different strains of rats. A number of factors, such as diet, age of animals, and rate of spontaneous tumor formation, have to be considered in evaluation of the results. Engel and Copeland,[2] using a semipurified diet, have reported the development of mammary tumors in 74% of females of an Alabama E. S. strain of rats. They believe that the rate of breast-tumor formation with 2-AAF can be influenced by diet, and ascribe the high incidence of tumors in their colony to some dietary deficiency in semipurified diets.[3] In other reports the incidence of breast tumors ranges from 64% (23 of 36) in a Wistar strain of rats [4] to 4% (1 in 25) in a piebald strain.[5] Mammary tumors produced by 2-AAF are also influenced by hormonal stimulation. Bielschowsky [4] has demonstrated that oophorectomy decreased the incidence of tumors from 64 to 9%. As far as the

This work was supported by a Grant-in-Aid from the National Cancer Institute of Canada.

From the Department of Pathology, St. Mary's Hospital, and the Departments of Experimental Surgery and Pathology, McGill University.

Dr. Ross is now with the Department of Pathology, St. Michael's Hospital, Toronto, Canada. Dr. Skoryna is a Senior Research Fellow, National Cancer Institute of Canada.

1. Skoryna, S. C.; Ross, R. C., and Rudis, L. A.: Histogenesis of Sebaceous Gland Carcinomas Produced in Rats by 2-Acetylaminofluorene, J. Exper. Med. **94**:1-8, 1951.

2. Engel, R. W., and Copeland, D. H.: Influence of Diet on the Relative Incidence of Eye, Mammary, Ear Duct, and Liver Tumors in Rats Fed 2-Acetylaminofluorene, Cancer Res. **11**: 180-183, 1951.

3. Engel, R. W., and Copeland, D. H.: Protective Action of Stock Diets Against the Cancer-Inducing Action of 2-Acetylaminofluorene in Rats, Cancer Res. **12**:211-215, 1952.

4. Bielschowsky, F.: Distant Tumors Produced by 2-Amino and 2-Acetyl-Amino-Fluorene, Brit. J. Exper. Path. **25**:1-4, 1944.

5. Bielschowsky, F.: Comparison of the Tumors Produced by 2-Acetyl-Amino-Fluorene in Piebald and Wistar Rats, Brit. J. Exper. Path. **27**:135-139, 1946.

relationship to spontaneous breast tumors is concerned, Bonser [6] found that in mice the differences in genesis between spontaneous, hormone-induced, and chemically induced mammary cancer were of degree rather than of kind.

EXPERIMENTAL METHODS

Female rats were taken from a colony of a hooded strain from the University Clinic of the Royal Victoria Hospital, inbred since 1933. The compound 2-acetylaminofluorene was added to purina® meal diet in a concentration of 0.04%. Administration was started at 15 weeks of age and continued for the subsequent 11 months.

As a routine, animals were killed in the terminal stage and breast tumors were removed for sections, although some of the mammary glands were excised surgically while the lesions were still small. Part of the pathological material discussed was taken from rats that died during the experiment as a result of intercurrent pulmonary infections. Routine examination with hematoxylin and eosin and Masson trichrome stains was made of all mammary glands in which tumors developed.

GROSS PATHOLOGY OF TUMORS

Gross mammary tumors developed in 17% of the animals, as compared with an incidence of 5% of spontaneous tumor formation in this colony. Multiple tumors developed in two or more mammary glands in 21% of the animals. Tumors were first observed after five and one-half months of administration of the 2-AAF. Some of the tumors appeared as late as 18 months after the beginning of the experiment.

In the early stages, the tumors appeared as subcutaneous nodules which were freely movable under the skin as well as against the chest wall. Later, fixation to the skin was frequently observed, although the tumors invariably remained mobile over the chest wall. Ulceration, with discharge of necrotic material, was a frequent feature in large tumors.

The majority of tumors grew rapidly, in two to three weeks attaining the size of a large olive. The largest tumor observed was 5 cm. in diameter. In the case of larger tumors in the flexion creases of the extremities, the animals were frequently disabled. On blunt dissection the tumors usually could be readily peeled out as firm, oval, lobulated nodules. The cut surface was pinkish white with a lobular pattern.

MICROSCOPIC OBSERVATIONS

Normal Breast.—Histologically the normal virgin rat breast consisted of scattered small clusters of acini adjacent to small ducts. These lobules were located superficial and deep to the layer of striated muscle which was found in the subcutaneous tissue (Fig. 1*A*). Surrounding the ducts and acini there was a minimal amount of intralobular connective tissue. The lobules were separated by loose fibroadipose tissue in which small nerves were numerous, especially near the nipple. Occasional mast cells were present in the connective tissue, as well as neutrophilic and eosinophilic leucocytes. In some cases mast cells and polymorphs were numerous, the mast cells commonly occurring adjacent to capillaries. In a few breasts marked chronic inflammation was evident, with numerous histiocytes, plasma cells,

6. Bonser, G. M.: A Comparison of the Evolution of Spontaneous and Chemically Induced Mammary Cancer in Mice, Acta, Union internat. contre cancer **6**:595-601, 1949.

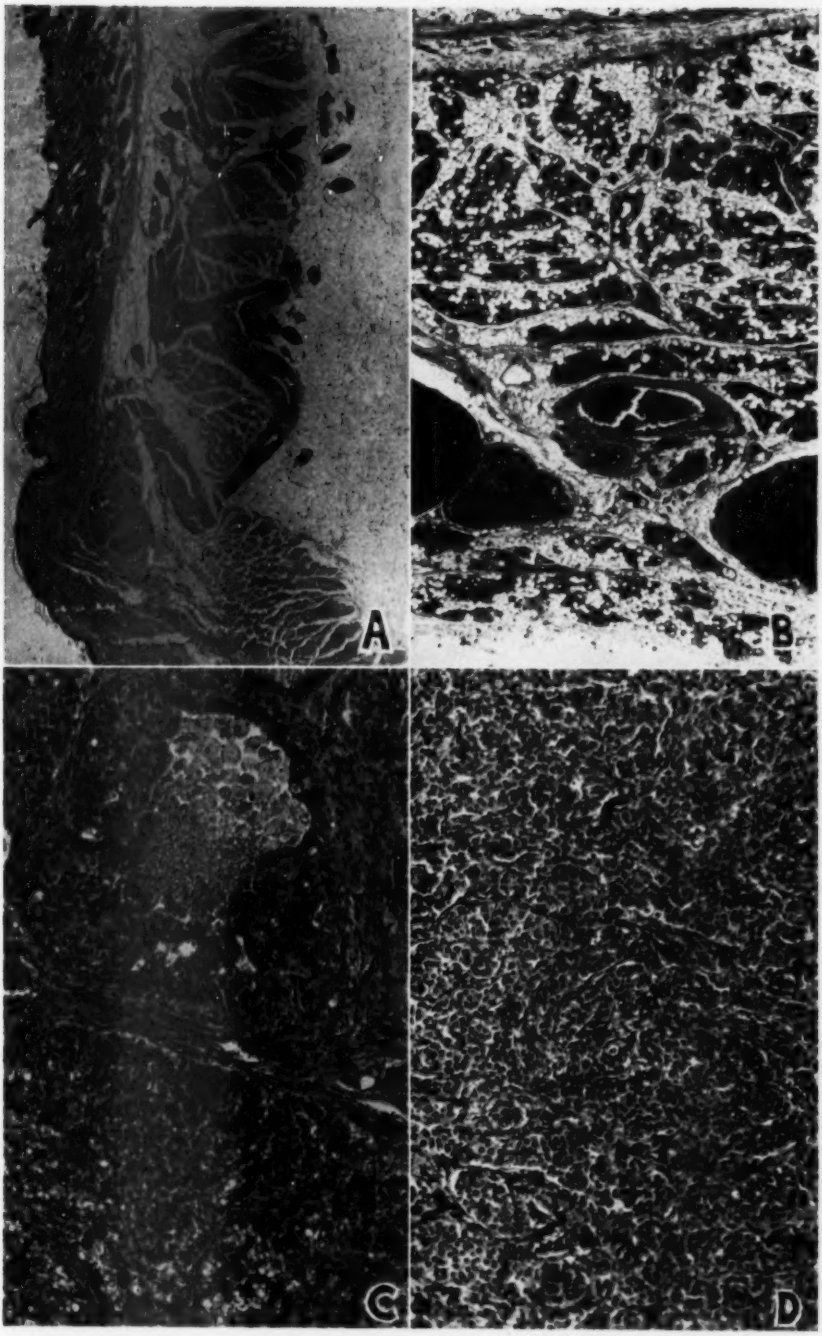


Fig. 1.—*A*, breast in a virgin rat which did not receive 2-AAF, showing small clusters of acini and ducts superficial and deep to the muscle. Hematoxylin and eosin; × 25. *B*, breast in bred rat which did not receive 2-AAF, showing acinar hyperplasia and also lymph nodes embedded in breast tissue. Hematoxylin and eosin; × 25. *C*, duct and acinar components in rat which did not receive 2-AAF. Hematoxylin and eosin; × 100. *D*, typical infiltrating duct carcinoma in rat which received 2-AAF.

lymphocytes, and scattered eosinophiles, neutrophiles, and mast cells. Not infrequently one or two lymph nodes were found between the superficial and deep portions of breast tissue (Fig. 1*B*).

In the virgin rat, the acinar epithelium might contain an occasional vacuole and the ducts a small amount of inspissated secretions. The epithelial cells contained a variable amount of finely granular, greenish-brown pigment, which had the staining reaction of melanin. In bred rats there was marked acinar hyperplasia, so that interlobular space was virtually eliminated, the majority of acinar cells were vacuolated, and the lumina contained secretion (Fig. 1*B*).

Breast Tumors.—In tumors occurring spontaneously or following the administration of 2-AAF, the characteristic histological picture was one of composite neoplasia of ducts, acini, and stroma. One of these components might be dominant, but it was usual for all three to be represented.

Duct Component: The neoplastic ducts ranged from simple ducts lined by a single layer of cuboidal epithelium to large solid masses of tumor cells similar to those observed in human duct carcinoma. In well-preserved areas the characteristic picture was one of large ducts lined by a thick layer of tumor cells in which secondary acini were evident. Centrally the duct usually contained inspissated secretion, lipophages, or degenerating tumor cells reproducing the picture of human comedo carcinoma (Fig. 1*C*). Alternatively the tumor might be composed of solid cords of neoplastic cells of a duct type similar to that seen in the usual human infiltrating duct carcinoma (Fig. 1*D*). The epithelial cells showed a variable degree of pleomorphism, and in those showing more nuclear variation mitotic figures were not infrequent.

Another variant of the duct component of tumors, seen to best advantage in smaller lesions, was the papillary type. This was similar to the intraductal papilloma of human pathology and its malignant equivalent. In this type papillary masses of tumor projected into dilated ducts, and, in addition, more solid masses of tumor were evident. Even in these small lesions the acinar and stromal components were usually evident.

Occasional tumors had foci of squamous metaplasia (Fig. 2*A*). These areas showed gradual transition from typical duct carcinoma to full-fledged keratinizing squamous carcinoma. In one of these there was a small area of sebaceous metaplasia.

Acinar Component: Of approximately equal prominence in most breast tumors was the acinar component. This consisted of closely spaced acini lined by a single layer of cuboidal cells with foamy or vacuolated cytoplasm (Fig. 2*B*). The vacuoles were frequently so large that they had led to distortion or rupture of the cell. The acini were fairly uniform in size, and their lumina frequently contained vacuolated eosinophilic secretion. Neither the intracellular vacuoles nor the secretion in the acini was mucicarminophilic. The acini were closely spaced, with scant stroma between individual scini and larger fibrous septa separating acinar groups. There was moderate nuclear variation, and mitotic figures were seen infrequently. The transition from acinar hyperplasia to neoplasia was gradual. Although the duct and acinar type of tumor were readily distinguished, in some areas a single duct might show transition from one type to the other (Fig. 2*C*).

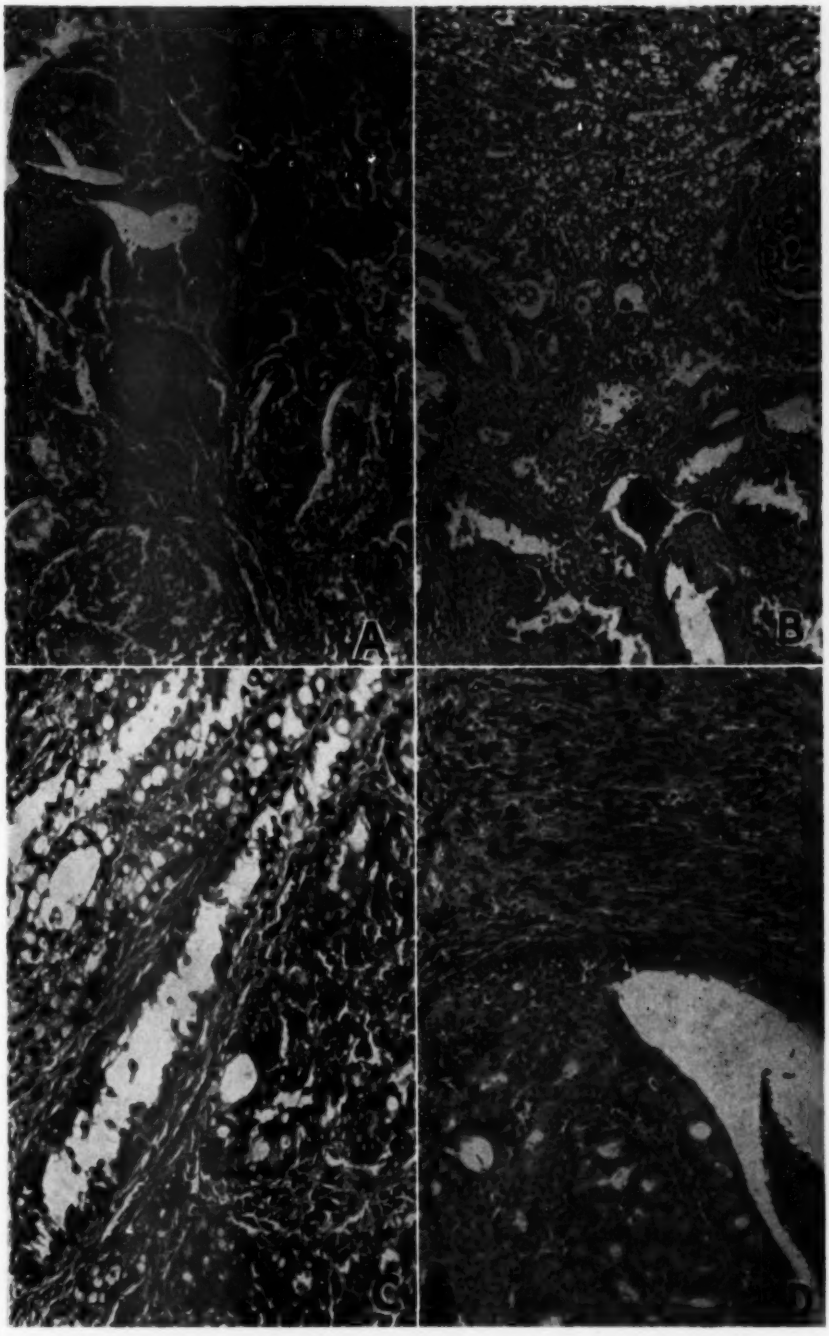


Fig. 2.—*A*, squamous metaplasia in tumor in rat which received 2-AAF. Hematoxylin and eosin; × 100. *B*, poorly preserved tumor showing duct, acinar, and stromal components in rat which received 2-AAF. Hematoxylin and eosin; × 100. *C*, poorly preserved tumor showing transition from duct to acinar type of rat which received 2-AAF. Hematoxylin and eosin; × 200. *D*, duct carcinoma and malignant stroma (carcinosarcoma) in rat which received 2-AAF. Hematoxylin and eosin; × 100.

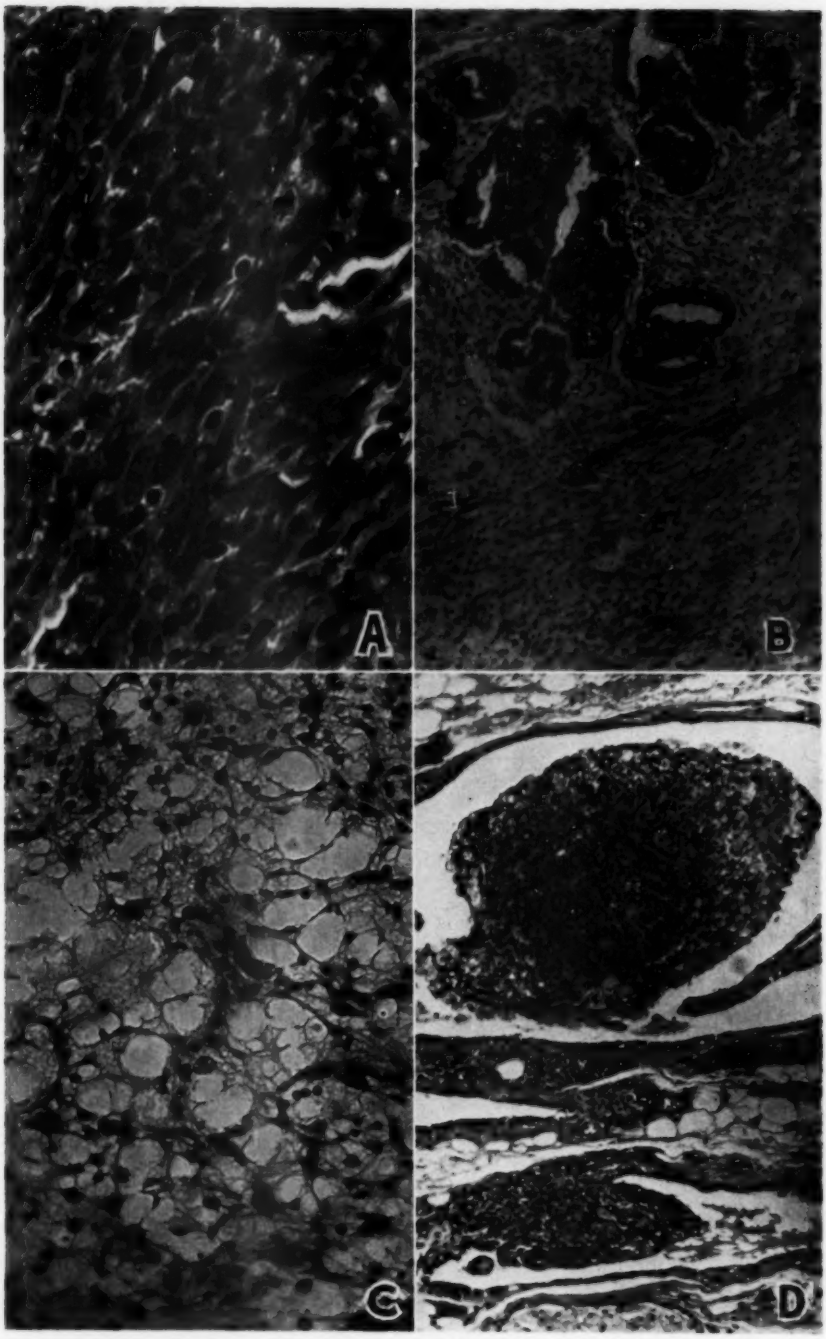


Fig. 3.—Same tumor as that seen in Figure 2 *D*. *A*, pleomorphic stroma. Hematoxylin and eosin; × 400. *B*, glandular tumor and myxomatous stroma. Hematoxylin and eosin; × 100. *C*, myxomatous stroma suggesting liposarcoma. Hematoxylin and eosin; × 190. *D*, myxomatous stroma bulging into ducts. Hematoxylin and eosin; × 100.

The Stroma: In general the stroma was cellular. It was scant between individual acini but formed septa between groups of acini and was more plentiful in duct areas of the tumor, either separating tumor masses or forming the core of papillary projections (Fig. 2*B*). In addition, larger blunt projections, consisting of stroma lined by epithelium, protruded into dilated ducts, as in intracanalicular fibroadenomas. The stroma, however, was more cellular and exhibited more nuclear variation than is seen in fibroadenomas. The picture was much more reminiscent of that seen in cystosarcoma phylloides of the human breast. The range of variability was from benign cellular connective tissue to pleomorphic fibrosarcoma (Figs. 2*D* and 3*A*).

Carcinosarcoma: In an unusual tumor developing in a rat receiving 2-AAF, the coexistence of carcinoma and sarcoma was particularly impressive. The epithelial component varied from typical comedo and infiltrating duct carcinoma (Figs. 1, *D* and 2 *D*) to anaplastic spindle-cell carcinoma which tended to blend with the stroma. In one area there was glandular carcinoma similar to human adenocarcinoma of the large bowel (Fig. 3*B*). The stromal component was highly malignant, and the picture was chiefly one of pleomorphic cellular fibrosarcoma (Figs. 2*D* and 3*A*). In other areas the malignant spindle cells were disposed in a more myxomatous fashion, and their foamy cytoplasm suggested liposarcoma (Fig. 3*C*). Tissue of this type was also evident within several ducts and in spite of its location was considered to represent malignant stroma rather than epithelium (Fig. 3*D*).

General Characteristics of Tumors: The periphery of the tumors was usually fairly well demarcated from the adjoining breast tissue. Infiltration of striated muscle was common. Infiltration was facilitated by the fact that normal breast tissue occurs both superficial and deep to striated muscle. Particularly in the larger tumors, areas of necrosis were frequent, and in many the necrotic tissue was undergoing peripheral organization. The larger tumors extended into the corium and produced bulging, atrophy, or ulceration of the overlying epidermis. The tumors were roughly spherical but had a distinct lobular pattern. This was due to compartmentation of the tumor by fibrous septa and also to the fibroadenomatoid bulging into dilated ducts. Although many of the tumors were considered to be malignant on histological grounds, no metastases, either local or distant, were demonstrated.

Fibroadenomas: Another common tumor was the fibroadenoma, which was usually pericanalicular in type. Characteristically the stroma was relatively acellular and often hyalinized, and the epithelium was inactive. In some the stroma consisted of closely spaced coarse collagen bundles. Scattered mast cells were evident, and fat was occasionally prominent in the connective tissue. No transitions between fibroadenomas and tumors with more cellular stroma were observed.

COMMENT

In the strain of rats used in this study tumors of the mammary gland were mixed in type.

One group consisted of fibroadenomas, similar to those which occur in human breasts. These occurred spontaneously and in rats which received 2-AAF. The fibroadenomas were chiefly pericanalicular, and the stroma was often hyalinized and the epithelium quiescent.

The other group accounted for the majority of the large tumors, and these were of composite origin. The epithelium of both ducts and acini and to a variable extent the stroma were neoplastic. Tumors of this type developed spontaneously and in rats to which 2-AAF had been administered. However, the fully developed lesion, carcinosarcoma, occurred only in a rat which received carcinogen.

In these tumors the duct component was an accurate reproduction of similar tumors in the human breast, ranging from benign intraductal papillomas to frank infiltrating duct carcinoma. The acinar portion of the tumor differed from its human counterpart, lobular carcinoma. Evidence of secretion was much greater and the transition from hyperplasia to neoplasia more gradual. This difference may well have been due to the preexisting acinar hyperplasia resulting from breeding of the rats.

The stromal changes are of particular interest and may be compared to those seen in cystosarcoma phyllodes in the human. However, the parallel cannot be drawn very closely, since in cystosarcoma the stromal lesion is the central theme, whereas in the rat it is usually subsidiary.

Although both types of tumor in the rat involved connective tissue as well as epithelium, no evidence of any relation between fibroadenomas and the other composite tumors was observed.

SUMMARY

1. In hooded female rats the incidence of breast tumors was increased threefold by the administration of 2-AAF.

2. Breast tumors were either benign fibroadenomas or neoplasms to which duct and acinar epithelium as well as stroma contributed.

3. The extreme example of the latter tumor, a carcinosarcoma, was seen only in a rat which received carcinogen.

4. The fibroadenomas were analogous to those seen in the human breast, but the other tumors, although having many of the features of human breast lesions, have no true human counterpart.

5. Although in both types of tumors epithelium and stroma were components, no transitions from benign fibroadenomas to the more malignant tumors were observed.

POLYCEREBROSIDES IN GAUCHER'S DISEASE

I. Isolation, Composition, and Physical Properties

L. LAHUT UZMAN, M.D.
BOSTON

INVESTIGATIONS on water-soluble glycolipid constituents of tissue undoubtedly owe their impetus in recent years to the isolation by Klenk [1] of a class of lipids named gangliosides and to the relationship these complex compounds were heralded to bear to the "storage substances" accumulating in various organs in Tay-Sachs disease,[1a,c] Niemann-Pick disease,[2] and gargoylism.[3] Although the gangliosides were first isolated from the brains of patients who had succumbed to Tay-Sachs disease and were so named because they were considered peculiar to nerve cells in this disease, subsequent work has shown these compounds to be present in normal brain [1a,c] and in increased amounts in the brain in Niemann-Pick disease with cerebral involvement.[4] In addition, they are present as minor constituents of other normal tissues.[5] Brante [3] recently presented strong evidence to support the belief that gangliosides actually may be present in much greater amounts in white matter than in the neuronal bodies themselves, a conclusion that Klenk himself seems to have reached in his study of the brain in Niemann-Pick disease.[6] However, the inconsistency with which the enrichment in gangliosides appears to occur in these diseases [7] and the probability that other glycolipids, similar in solubility characteristics but varying considerably in composition, may be present have rendered a proper evaluation of their significance difficult. Thus, in subsequent studies, Klenk failed to find an increase in gangliosides in organs from persons with the juvenile form of Tay-Sachs disease, and, similarly, a significant enrichment in gangliosides, as measured by the neuraminic acid content of different tissues, was lacking in one person who had Niemann-Pick disease.[6] Since gangliosides by no means appear to be substances of homogeneous composition,[8] it has become necessary to define these compounds at present as constituting a class of water-soluble

From the Department of Pathology, Harvard Medical School, and the Chemical Pathology Laboratories of The Children's Cancer Research Foundation, The Children's Medical Center.

1. Klenk, E.: (*a*) Ztschr. physiol. Chem. **273**:76, 1942; (*b*) ibid. **262**:128, 1939; (*c*) Ber. deutsch. chem. Gesellsch. **75**:1632, 1942; (*d*) Ztschr. physiol. Chem. **235**:24, 1935.

2. (*a*) Tropp, C., and Eckardt, B.: Ztschr. physiol. Chem. **245**:163, 1937. (*b*) Klenk.[1b,d]

3. Brante, G.: Fette u. Seifen **53**:457, 1951.

4. Klenk.[1d] Tropp and Eckardt.[2a]

5. Klenk, E., and Rennkamp, F.: Ztschr. physiol. Chem. **273**:253, 1942.

6. Klenk, E.: Ztschr. physiol. Chem. **282**:84, 1947.

7. Tropp and Eckardt.[2a] Klenk.[6]

8. Klenk, E.: Ztschr. physiol. Chem. **267**:128, 1940. Klenk.[1c] Klenk and Rennkamp.[5]

glycolipids characterized by their high content of neuraminic acid (21 to 24%); the structure of the latter is as yet undetermined, but it is believed to be a hexosamine.[9]

More recently, Folch and his associates [10] isolated another type of water-soluble glycolipid from normal mammalian brain. The name "strandin" was given to this lipid because of the tendency toward formation of highly oriented strands on evaporation of an aqueous solution of the material. Strandin, though similar to Klenk's gangliosides in having fatty acids, hexose, and sphingosine or sphingosine-like residues in its composition, differs from the latter in its low content of neuraminic acid (1.5%) and by the presence of certain chromogens and of certain nitrogenous compounds released on hydrolysis and appearing in the water-soluble amino nitrogen fraction. It is noteworthy that strandin normally occurs in considerable amounts only in brain, less than 0.01% being present in other organs studied.

The present investigations were undertaken with the intent of ascertaining what glycolipids, other than the cerebroside kerasin, are stored in the organs of patients with Gaucher's disease. In a previous communication [11] it was shown by the author that kerasin is found in spleens from patients with Gaucher's disease in the form of a rather unique lipoprotein (the Gaucher lipoprotein—GLP) and that the lipid moiety of this lipoprotein accounted for about 70% of the lipid-carbohydrate present in the hydrolysates of total lipid extracts obtained from these organs. Although, in general, the nonquantitative nature of isolation procedures may justify the assumption that all the lipid-hexose content of the spleen in Gaucher's disease should be ascribed to the Gaucher lipoprotein, a search for the presence of other, hitherto unknown cerebrosides appeared indicated, inasmuch as the possibility of the accumulation of glycolipids of the gangliosides type in this disease, in which the formation of stable cerebroside-protein complexes appears to be a main feature of the chemical pathogenesis, could not be excluded. Investigations in my laboratory have culminated in the isolation of a water-soluble glycolipid from organs of patients having Gaucher's disease. This glycolipid contains hexose as part of its molecular structure but differs from the gangliosides reported previously. Because of the recovery of cerebroside from its partial hydrolysis products, the name "polycerebroside" has been given to this glycolipid. The present report deals with the isolation and some of the chemical and physical properties of this water-soluble lipid fraction. The interesting properties which aqueous solutions of this material exhibit in terms of forming stable complexes with proteins in vitro will be the subject of a later communication.[12]

ISOLATION

Materials.—The organs employed in this study had the following sources:

Case 1: Surgically removed fresh spleen, and brain obtained at postmortem examination six weeks later, from a 5-month-old boy with infantile Gaucher's disease. There was generalized involvement of all organs, including lung and brain.

9. Blix, G.; Svennerholm, L., and Werner, I.: Acta chem. scandinav. **6**:358, 1952. Blix, G.: ibid. **2**:467, 1948. Klenk, E.: Ztschr. physiol. Chem. **268**:50, 1941. Blix, G.: Scandinav. Arch Physiol. **80**:46, 1938.

10. Folch, J.; Arsove, S., and Meath, J. A.: J. Biol. Chem. **191**:819, 1951.

11. Uzman, L. L.: A. M. A. Arch. Path. **51**:329, 1951.

12. Uzman, L. L.: Unpublished data.

Case 2: Surgically-removed, formalin-fixed spleen from a 2-year-old girl with Gaucher's disease. The spleen was finely minced and freeze-dried, the dry powder being suspended in water (3 ml. water per gram of powder) just before being processed.

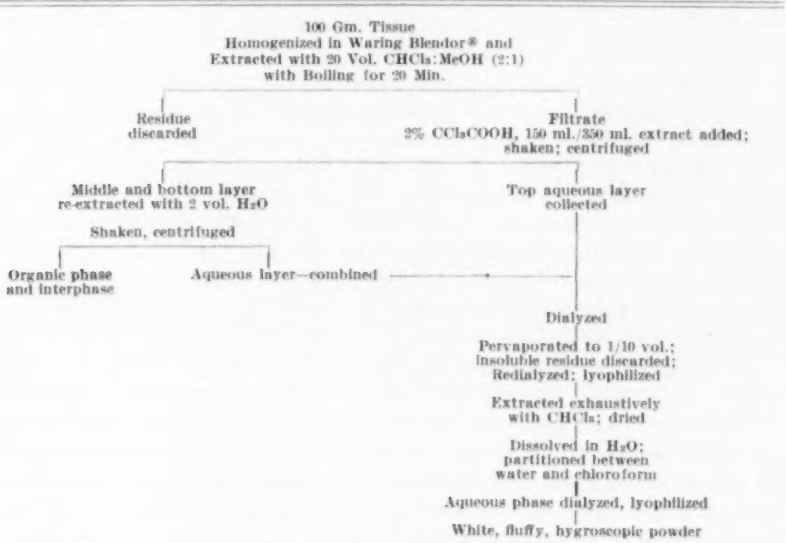
Case 3: Surgically removed fresh spleen from an 11-year-old girl with Gaucher's disease.

Case 4: Surgically-removed fresh spleen from a 34-year-old woman with the adult form of Gaucher's disease.

All organs were frozen solid in an ice chest (solid carbon dioxide) immediately after removal and were stored in this state until they were processed.

Procedures.—An outline of the procedure used in isolation of the polycerebroside is given in the accompanying Table. The procedure depends essentially on

Procedure Used in Isolation of Polycerebroside from Organs of Patients with Gaucher's Disease



dissociation of the lipid from cytoplasmic proteins by treatment of tissue with a mixture of hot chloroform-methanol and on subsequent separation of the polycerebroside from other lipids present in the extract (including cerebrosides) by virtue of the markedly greater solubility of the polycerebroside in acidified aqueous solvents. Just prior to being extracted, the organs were allowed to thaw at room temperature until they were soft enough to be cut with a knife. At this stage, 100-gm. portions were cut off and homogenized in 25-gm. batches with 20 vol. of a 2:1 mixture of chloroform-methanol [13] in a Waring blendor.® The combined homogenates were boiled for 20 minutes in a water bath with violent shaking.

13. Folch, J.; Ascoli, I.; Lees, M.; Meath, J. A. and LeBaron, F. N.: J. Biol. Chem. **191**:833, 1951.

cooled immediately, and filtered on a Buchner funnel with suction. It was found that if the extract was left in contact with the tissue residue for any length of time before filtration, the amount of the polycerebroside recovered was small, presumably because of the *de novo* formation of polycerebroside-protein complexes. The filtered chloroform-methanol extracts were then placed in stoppered, graduated cylinders and allowed to equilibrate in a cold room at 2 C. From this point on, all operations were carried out in the cold. To each 350-ml. portion of extract, 150 ml. of 2% trichloroacetic acid was added. The two phases were allowed to form and then were again disrupted by intermittent shaking over a period of one and a half hours. At the end of this time, the aqueous layer was separated from the organic layer by centrifugation, and the aqueous acid layer was carefully siphoned off without disrupting the white fluffy interphase layer. The combined bottom and interphase layers were then extracted with 2 vol. of water by vigorous shaking for two hours in the cold, allowed to stand for two hours, with resolution of the two phases, and shaken again for two hours. The aqueous layer was separated by centrifugation and siphoned off. The trichloroacetic extract was now combined with the aqueous extract and dialyzed for 24 hours against large volumes of distilled water.

It should be emphasized here that the aqueous extraction following the trichloroacetic acid extraction is not a step that is introduced to complete the extraction of the polycerebroside from the organic phase, but rather constitutes an essential part of the isolation procedure, inasmuch as the bulk (more than 60%) of the polycerebroside is carried in this fraction. Pilot experiments indicated that the initial trichloroacetic acid extraction served mainly to prevent the fixation of the polycerebroside in the bulky interphase layer between the organic and aqueous phases. Dialysis had to succeed the previous steps as soon as possible to prevent hydrolytic cleavage of the polycerebroside by the acid, inasmuch as partial degradation seems to proceed, even in the cold, on prolonged exposure to trichloroacetic acid.

The dialyzed solutions presented a slightly opalescent appearance. Any insoluble residue was removed by centrifugation. The solutions were then pervaporated in dialysis bags in a strong current of air to about one-tenth their original volume. The insoluble residue appearing on concentration was found to consist mostly of phosphatides, and these were separated by centrifugation and discarded. The pervaporated solutions were redialyzed against water and lyophilized. The product obtained on lyophilization was a slightly yellow, highly hygroscopic powder. The powder was repeatedly extracted with chloroform at room temperature by suspending the material in the organic solvent, shaking, and centrifuging. The chloroform supernatant was found to extract more phosphatides (molar N/P ratio in supernatant: 1.01). The elimination of contaminating phosphatides was completed by dissolving the whitish powder in water and partitioning it between water and chloroform. The aqueous phase was siphoned off after centrifuging of the biphasic mixture, redialyzed for 24 hours against distilled water, and lyophilized. The resulting product was a white, fluffy, hygroscopic powder. It was soluble in water and 2 to 4% trichloroacetic acid and proved insoluble in chloroform, chloroform-methanol, methanol, ethanol, and benzene. However, if a mixture of chloroform-methanol (2:1) was added in amounts barely sufficient to yield a monophasic mixture to an aqueous solution of the polycerebroside, the material could be kept in

solution. Drops of aqueous solutions spread onto glass microscope slides and allowed to dry produced uniform, transparent, continuous, resinous films that could be removed in brittle flakes. Examination under polarized light showed no evidence of orientation.

Yield.—The yield in Case 1 was: spleen, 176 mg., and brain, 264 mg., per 100 gm. of fresh tissue. The yield in Case 2 was 482 mg. per 100 gm. of dry tissue; in Case 3, 49 mg. per 100 gm. of fresh tissue, and in Case 4, 62 mg. per 100 gm. of fresh tissue.

CHEMICAL COMPOSITION

Elementary analysis of the material gave the following results: carbon, 52.89%; hydrogen, 8.11%; nitrogen, 4.02%; sulfur, 0.0%, and phosphorus, 0.02%. However, some of the preparations had a phosphorus content as high as 0.2%. There was no free amino nitrogen prior to hydrolysis. Acid hydrolysis in 6N hydrochloric acid, carried out in sealed tubes at 110 C. for eight hours, gave an amino nitrogen value of 3.94%. Partition of the acid hydrolysis products between water and chloroform showed the amount of water-soluble nitrogen to be 0.02%, while the amount of chloroform-soluble nitrogen was 3.9%. This observation indicated that practically all the nitrogen content of the polycerebroside probably could be ascribed to sphingosine or to a sphingosine-like compound. The hexose content was found to be 18.4% (estimated as galactose). Fermentation studies with glucose-fermenting and galactose-fermenting strains of yeast indicated that in the polycerebroside preparations obtained from brain, the hexose consisted almost wholly of galactose, while in the polycerebroside from splenic sources, the hexose moiety consisted of a mixture of galactose and glucose in a ratio of 4:1. Baryta hydrolysis of the polycerebroside, effected with a saturated solution of barium hydroxide in sealed tubes at 110 C. for two hours, yielded the barium salts of the fatty acids present. The insoluble barium salts were separated from the hydrolysate by centrifugation. They were then suspended in dilute sulfuric acid, and the fatty acids were liberated by heating the suspension for 40 minutes in a boiling-water bath. The barium sulfate precipitate was centrifuged off and washed with dilute methanolic sulfuric acid (5% sulfuric acid in 80% methanol), and the washing was added to the supernatant, which was then concentrated to a thick syrup *in vacuo*. The fatty acids were obtained in crystalline form (fine amber-colored needles) from the methanolic solution. The fatty acid yield accounted for 25% of the polycerebroside by weight (M.P., 91 C., copper block, uncorrected; neutral equivalent, 375). Hydrolysis of the polycerebroside with 2N sulfuric acid in a boiling-water bath for one hour resulted in the formation of a black insoluble precipitate. The fatty acids were isolated from the hydrolysate as the silver salts and were subjected to purification by treatment with methanolic hydrochloric acid (1.0 N) and removal of the silver chloride formed. The fatty acids obtained in this manner accounted for 22% of the polycerebroside by weight (M.P., 81 C., copper block, uncorrected; neutral equivalent, 330; iodine number, 2). The differences observed in the melting point of the fatty acid fractions thus obtained by two different types of hydrolysis appeared to indicate that the fatty acid moiety consisted of a mixture of fatty acids, acid hydrolysis under the conditions employed resulting in some degradative changes or losses occurring through adsorption onto the black, insoluble precipitate.

Supernatants of the baryta hydrolysate were used for choline estimations.[14] However, neither baryta hydrolysis nor hydrolysis with alcoholic sodium hydroxide, according to Brante, yielded any material which could be precipitated as reineckate. Inasmuch as the formation of a black precipitate on treatment with mineral acids has been noted by Walz [15] and described by Klenk [16] as a property of gangliosides, the possibility of the presence of neuraminic acid had to be investigated. Application of the procedure employed by Klenk and Langerbeins [16] for estimation of neuraminic acid with Bial's reagent, however, proved this substance to be absent, resulting only in development of the green color due to galactose. Because of the similarities in extraction procedure and composition that the polycerebroside bears to the glycolipid "strandin," a sample was analyzed by Dr. J. Folch with his method[10] for determination of the chromogenic material. By this procedure the polycerebroside was found to contain 55% of the "chromogen" content of strandin.

The iodine number of the polycerebroside estimated after treatment with Hübl's reagent was found to be 20.2. This value would correspond to that expected if all the chloroform-soluble nitrogen obtained on acid hydrolysis of the polycerebroside was assigned to sphingosine. The absence of glucosamine was confirmed by paper partition chromatography of the lipid hydrolysates.

Samples of polycerebroside were hydrolyzed in sealed tubes with 6N hydrochloric acid at 110 C. for 14 hours. The acid was removed by repeatedly taking the hydrolysate to dryness *in vacuo*. Appropriate aliquots were then subjected to paper partition chromatography, using phenol-water as the solvent system. A faint ninhydrin-positive spot was detectable in some chromatograms that had not been dried in an oven or allowed to dry too long in air. However, this spot did not correspond to any known amino acid or to glucosamine, the latter being used as a control, either parallel to the hydrolysate or added to an aliquot of hydrolysate that was to be chromatographed. The disappearance of this ninhydrin-positive spot after prolonged drying indicated that it was volatile or easily destroyed by oxidative degradation. The limited amount of material available made it impossible to pursue determination of its identity any further at that time.

The analytical data presented here indicate the isolated material to be a water-soluble, undialyzable glycolipid of the cerebroside class, containing hexose, fatty acids, and a chloroform-soluble nitrogenous compound of the sphingosine class. Release of free amino groups after acid hydrolysis was taken to indicate that the nitrogen probably constituted an amide linkage with a fatty acid. This supposition was confirmed by the characteristics of its infrared absorption spectra, which will be presented later. Therefore, it appeared of particular interest to study the partial hydrolysis products of the material in an attempt at further elucidation of its structure. That partial hydrolysis did take place when the polycerebroside was allowed to stand for any length of time in 4% trichloroacetic acid was noted in the course of pilot experiments, and hence due care was exercised in the trichloroacetic acid treatment involved in the isolation procedure, as mentioned previously. The primary and most obvious evidences of hydrolytic degradation were the appearance of reduc-

14. Brante, G.: Acta physiol. scandinav. 1949, Vol. 18, Supp. 63, pp. 51.

15. Walz, E.: Ztschr. physiol. Chem. **166**:210, 1927.

16. Klenk, E., and Langerbeins, H.: Ztschr. physiol. Chem. **270**:185, 1941.

ing substances in the dialysate of a solution of the polycerebroside in 4% trichloroacetic acid which had been allowed to stand overnight at room temperature and neutralized immediately prior to dialysis and the eventual formation of a brownish flocculate in the nondialyzable fraction.

Because of this experience, partial hydrolysis of the polycerebroside was carried out by dissolving 85 mg. of lyophilized material in 4.0 ml. of 4% trichloroacetic acid in a stoppered centrifuge tube. This was allowed to stand at room temperature (26 C.) and was observed carefully for the first signs of the appearance of a flocculate. After 90 to 120 minutes, a whitish cloudiness formed, which shortly resolved itself into a fine flocculate. The flocculate was immediately centrifuged off, washed repeatedly with 1.0-ml. portions of water, and finally dissolved in 2.0 ml. of chloroform-ethanol (1:2). After it stood 24 hours in the cold room, a bulky white precipitate ensued, consisting of rosettes of highly birefringent fine needles. These were collected by filtration and dried in a desiccator. The yield was 12.8 mg., or 15% of the original material by weight. The selenite plate test[17] carried out after recrystallization from pyridine showed the crystals to be indistinguishable from kerasin. Analysis of this partial hydrolysis product indicated a nitrogen content of 1.94%, 21.0% hexose calculated as galactose, and no phosphorus. Of the material 0.1 mg. was dissolved in 100 μl. of chloroform-methanol (2:1), and various aliquots were delivered with calibrated micropipettes onto Whatman No. 1 filter paper sheets for paper partition chromatography of lipids, according to the method of Bevan and his associates.[18] The locations of the spots formed were detected by exposing the paper for a few minutes to iodine fumes. The material had the same fast-moving migration characteristics as samples of pure kerasin, with, however, a greater tendency toward "tailing." These data indicated that partial hydrolysis of the original water-soluble material would yield a cerebroside similar to kerasin in solubility, composition, and chromatographic behavior, though not necessarily identical with it. This compositional affinity to cerebrosides and the physical-chemical behavior of the material in aqueous solutions (to be described in the following sections), which definitely indicates a large molecular weight, appear to justify the designation of "polycerebroside" assigned to this water-soluble lipid fraction.

PHYSICAL PROPERTIES

Electrophoretic Behavior.—Because of its solubility in aqueous solvents, the polycerebroside lends itself admirably to electrophoretic studies in the Tiselius cell. This lipid fraction was found to behave homogeneously over a wide and biologically important range of pH. It was found to migrate as a single boundary in 0.1 M citrate buffer at pH 4.5, 0.1 M phosphate buffers at pH 6.5 and pH 7.5, and 0.1 M diethylbarbiturate buffer at pH 8.6 (Fig. 1). The calculated mobility in 0.1 M diethylbarbiturate at pH 8.6 was -9.72×10^{-4} cm^2/volt/sec. Thus, the polycerebroside in aqueous solution appears to behave like a polyacid with a negative net charge. In view of the absence of sulfur and the presence of phosphorus in only

17. Rosenheim, O.: Biochem. J. **8**:110, 1914.

18. Bevan, T. H.; Gregory, G. I.; Malkin, T., and Poole, A. G.: J. Chem. Soc., London, p. 841, 1951.

trace amounts, it would be reasonable to conclude that the presence of free carboxyl groups is responsible for its polyacid nature.

Diffusion Coefficient and Estimated Molecular Weight.—The diffusion characteristics of the polycerebroside were studied in the Tiselius cell, a compensator being used for initial adjustment of boundaries and free diffusion being allowed to proceed against distilled water. The diffusion constant was calculated [19] from the plotting of A^2/H^2 against (t), estimated from Philpot-Svensson diagrams obtained at regular intervals over 36 hours, and after temperature corrections was found to be

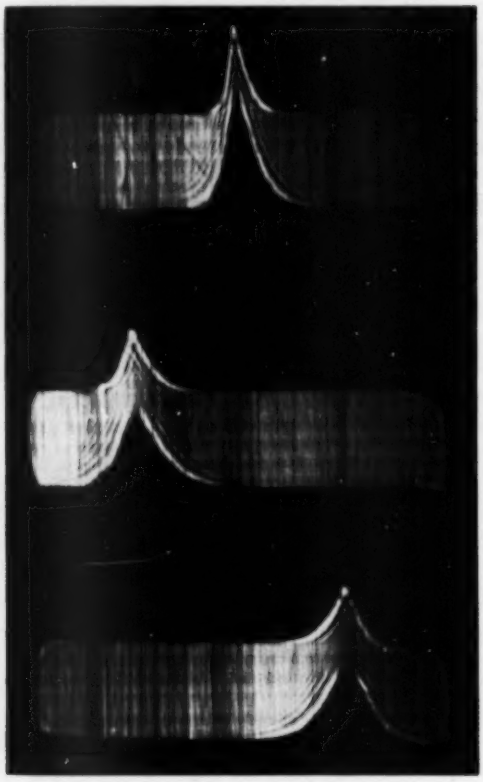


Fig. 1.—Patterns of electrophoretic runs of polycerebroside in Perkin-Elmer Tiselius electrophoresis apparatus. The 2.0-ml. cells were used. Time of run in all instances was 65 minutes; potential, 165 volts; current, 11.0 ma.; lipid concentration, 0.6%. Top, ascending pattern in 0.1 M citrate buffer at pH 4.5. High degree of opalescence of solution at this pH renders descending limb pattern unsuitable for photographic reproduction. Middle, ascending pattern of run in 0.1 M diethylbarbiturate buffer at pH 8.6. Bottom, descending pattern of run in 0.1 M diethylbarbiturate buffer at pH 8.6.

19. Wiener's equation was used: $D_{o,h} = \frac{\omega^2 A^2}{4\ tH^2}$, where t = elapsed time in seconds, ω = cm. in cell per enlargement unit on photographic plate, A = area enclosed by pattern, and H = maximum height of peak.

$D_{20} = 9.4 \times 10^{-7}$ cm.²/sec. The molecular weight,[20] estimated from the Stokes-Einstein equation, gave a value of 30,000. This figure was found to be in close agreement with the number-average molecular weight obtained from osmotic pressure measurements when 0.4, 0.8, 1.0, and 1.3% solutions of the polycerebroside in 0.1 M phosphate buffer at pH 6.5 were studied in a modified Zimm osmometer. Extrapolation of the (π/C,C) curve to infinite dilution indicated a number-average molecular weight of 32,000.

The agreement between the data from osmotic pressure measurements and diffusion studies was taken to indicate that the molecular shape of the polycerebroside presented no significant axial dyssymmetry. This assumption was supported by the fact that neutral aqueous solutions of the polycerebroside were not observed to show any birefringence of flow even under the influence of high shearing forces such as are brought into play in using the capillary method with application of increasing pressure gradients.

Treatment of the polycerebroside with alkali appeared to result in irreversible changes in its physical properties. Thus, when a 0.5% polycerebroside solution in 0.1 M diethylbarbiturate buffer at pH 8.6 was neutralized and dialyzed against distilled water, the aqueous solution of polycerebroside was converted into a gel, which on further standing at 2 C. was observed to become sediment as a fibrillar precipitate. The same phenomenon was noted after dissolving the polycerebroside in water, adding 0.1 N sodium hydroxide until the solution reached pH 8.5, neutralizing with 0.1 N hydrochloric acid, and dialyzing out the salt. Hence, in spite of the homogeneous electrophoretic behavior of the polycerebroside when it was studied at alkaline pH, it is difficult to escape the conclusion that drastic changes in the molecular arrangement do take place at higher pH levels. These changes would be compatible with the institution of strong electrostatic repulsive forces between negatively charged carboxyl groups situated adjacent to one another in the same molecule. Such a repulsion would result in uncoiling or unfolding of chain structures that go to make up the polycerebroside, producing marked molecular asymmetry. Once the ordered folding was thus disrupted by alkaline treatment, neutralization would result in only a random loose re-coiling, while the long relaxation time would favor the formation of a loose fibrillar network manifesting itself as a gel. Loss of interfibrillar water could then be expected to resolve this gel into a fibrillar precipitate, as actually observed in the course of these experiments. No change of composition and no release of free amino groups occurred as a result of alkali treatment. This observation would tend to attest the physical nature of the changes taking place in the course of such treatment.

---

20. According to the Stokes-Einstein equation (Einstein, A.: Ztschr. Elektrochem. **14**:235, 1908), the diffusion constant of a sphere of radius (r) cm. can be expressed as $D = \frac{RT}{6\pi\eta Nr}$, where η = coefficient of viscosity of medium in poises, $R = 8.3143 \times 10^7$ ergs/degree/mole, T = absolute temperature, and N = Avogadro's number (6.0228×10^{23}/mole). Since the weight of N spheres of radius r would be given by $\frac{4\pi}{3} N\rho r^3$, where (ρ) is the density of the solute (gm./cm.³), the molecular weight can be calculated if the diffusion coefficient is known as $M = \frac{R^3T^3\rho}{162\,\pi^2\eta^3 N^3 D^3}$. The frictional coefficient (f/f_o ratio) was neglected in this calculation.

Infrared Absorption Spectra.[21]—The spectral absorption characteristics of the polycerebroside in the infrared region were studied both with the intent of ascertaining the presence of an amide-type linkage, as indicated by the chemical analyses, and for comparison purposes with cerebrosides, to which the polycerebroside bears a close relationship in composition. Films of the polycerebroside were cast from an aqueous 1% solution onto silver chloride discs and allowed to dry in a desiccator over phosphorus pentoxide. A pure sample of strandin [22] was similarly cast. Pure kerasin, prepared by conventional methods from the spleen of a patient with Gaucher's disease, was examined in the form of a mineral oil mull. The infrared absorption spectrum of the polycerebroside is presented in Figure 2.

It can be observed that the material showed strong absorption bands at 3280 cm.$^{-1}$, 1645 cm.$^{-1}$, and 1545 cm.$^{-1}$, frequencies corresponding to the main absorption frequencies assigned to the -N-H stretching (3330 cm.$^{-1}$), C = 0 stretching

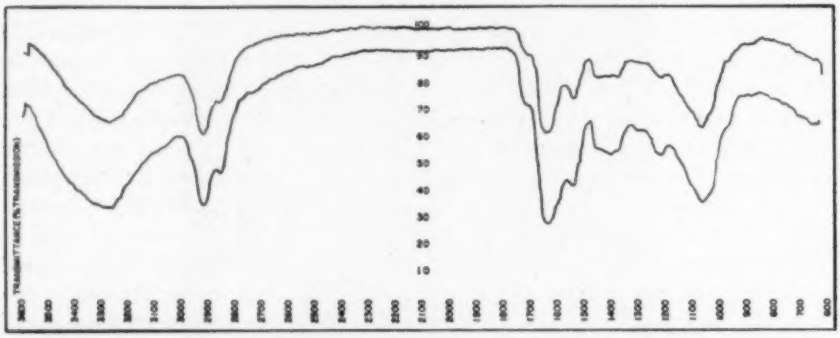


Fig. 2.—Infrared absorption spectrum of Gaucher polycerebroside obtained with Perkin-Elmer double-beam, self-recording infrared spectrophotometer, Model 21, NaCl prism. Films of different thicknesses were cast from aqueous solution onto silver chloride discs. Frequency is expressed in cm.$^{-1}$

(1670 cm.$^{-1}$), and N-H deformation (1,560 cm.$^{-1}$) of the amide group.[23] The weak absorption at 1725 cm.$^{-1}$ probably is due to unbonded C = 0 stretching of free carboxyl groups.

In recent years information accruing from work on the variations in the -NH frequency with hydrogen bonding of type -CO . . . HN-[24] has indicated that the absorption due to the perturbed NH frequency can appear in a wide range between 3440 cm.$^{-1}$ and 3070 cm.$^{-1}$, with the absorption of the unperturbed NH frequency being sharply located at 3440 cm.$^{-1}$

21. Dr. E. R. Blout performed these spectral studies and helped in the interpretation of the data.

22. Dr. J. Folch provided this purified sample of strandin.

23. Thompson, H. W.; Brattain, R. R.; Randall, H. M., and Rasmussen, R. S.: In Clarke, H. T.; Johnson, J. R., and Robinson, R. R.: The Chemistry of Penicillin, Princeton, N. J., Princeton University Press, 1949, Chap. 13.

24. Darmon, S. E., and Sutherland, G. B. B. M.: Nature, London **164**:440, 1949. Tsuboi, M.: Bull. Chem. Soc. Japan **22**:215, 1949; ibid. **22**:255, 1949. Mizushima, S.; Simanouti, T.; Nagakura, S.; Kuratani, K.; Tsuboi, M.; Baba, H., and Fujioka, O.: J. Am. Chem. Soc. **72**:3490, 1950.

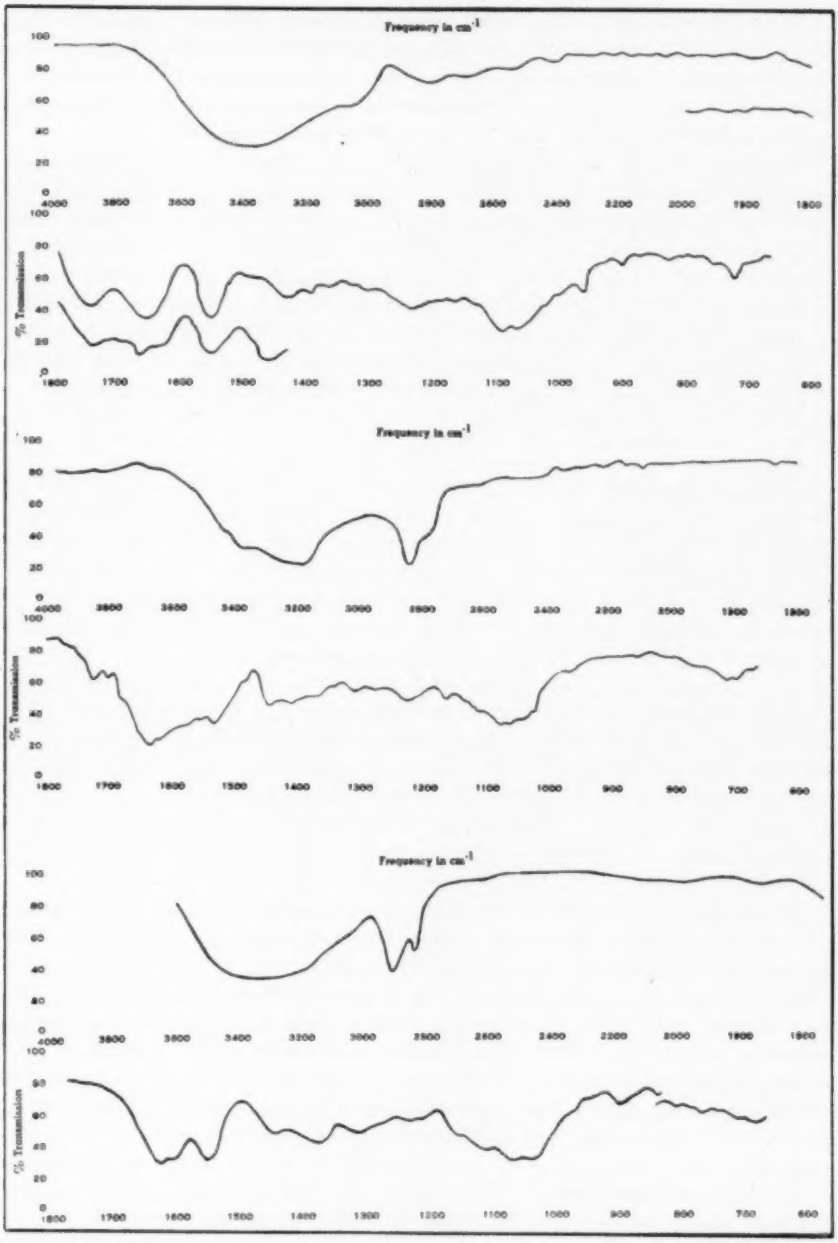


Fig. 3.—Infrared absorption spectra of kerasin, the Gaucher polycerebroside, and strandin recorded from the Perkin-Elmer single-beam infrared spectrophotometer, Model 12 C, NaCl prism. Frequency is expressed in cm.$^{-1}$ Top, kerasin from Gaucher spleen in form of mineral oil mull. Middle, polycerebroside from Gaucher spleen cast onto silver chloride disc from aqueous solution. Bottom, strandin from cattle brain cast onto silver chloride disc from aqueous solution.

It would seem that the broad band observed between 3100 cm.$^{-1}$ and 3450^{-1} can be ascribed to deformation of -NH of the amide through hydrogen bonding. The possibility also exists that this absorption may in part be contributed by the -OH. . . stretching (hydrogen-bonded). The latter consideration comes into foreplay in view of the known hexose content of the Gaucher polycerebroside.

Comparison of the infrared absorption spectra of kerasin, the Gaucher polycerebroside, and strandin (Fig. 3) shows certain absorption bands common to all. The most interesting at this juncture are the absorption bands at 1535 cm.$^{-1}$ and 1635 cm.$^{-1}$ in the polycerebroside, at 1550 cm.$^{-1}$ and 1625 cm.$^{-1}$ in the strandin, and at 1550 cm.$^{-1}$ and 1650 cm.$^{-1}$ in the kerasin spectra, ascribable to the C = 0 stretching and -NH stretching of the amide. Since kerasin is known to have all its nitrogen in the form of the amide link between lignoceric acid and sphingosine, this offers strong supportive evidence that both the Gaucher polycerebroside and strandin have part or all of their nitrogen in amide form. This evidence is in essential agreement with available analytical data.

COMMENT

Although the reader has been spared an account of the numerous pilot experiments that have shaped the features of the final isolation procedure presented, it will nevertheless be evident that the search for and isolation of the polycerebroside were conducted along lines that would be expected to yield a water-soluble glycolipid of the ganglioside or strandin class. Since the material isolated proved to belong to neither of these, it is presented as a new glycolipid occurring in organs of patients affected with Gaucher's disease. In naming this new lipid "polycerebroside" I was swayed by (1) its large molecular weight and macromolecular physical properties; (2) the close relationship observed in its composition to cerebrosides, its hydrolytic products being, in fact, mainly those one would obtain from the hydrolysis of a cerebroside, and (3) the fact that one of its partial hydrolysis products is undoubtedly a cerebroside. Perhaps it would be unnecessary to emphasize here that the prefix "poly" is in no way intended as designating a molecule composed solely of repeating, ordered, or homogeneous units of cerebrosides, although subsequent studies may show this actually to be the case.

The polycerebroside differs from Klenk's gangliosides by the absence of neuraminic acid or a related hexosamine. Since the gangliosides themselves have little homogeneity in their fatty acid constituents [25] or in their hexose composition,[8] a comparison with the polycerebroside in these respects would be unwarranted. The fatty acid moiety of the polycerebroside appears to be a mixture of C_{18} and C_{24} saturated fatty acids. Furthermore, though they seem to be undialyzable, further information on the particle size and physical behavior of the gangliosides in aqueous solution is lacking. Klenk [18] ascribed to them an equivalent weight of 1216 to 1510, from titration data. Assuming this weight to be a minimum contributed by the carboxyl group of neuraminic acid, as suggested by Klenk, the molecular weight would still be much smaller than that of the polycerebroside (approximately 30,000). On the other hand, the molecular weight given for strandin [10] is more than 250,000. Strandin also contains twice the amount of "chromogenic substance" by weight as the polycerebroside, although having only half the nitrogen content.

25. Klenk,[18,c] Klenk and Rennkamp.[5]

Furthermore, about half the strandin nitrogen is found in the water-soluble hydrolysis products, while all the nitrogen-containing substances in the polycerebroside are found in the chloroform phase. One would conclude, therefore, as was indicated also by the comparison of their infrared absorption spectra, that strandin and the polycerebroside described differ markedly in chemical composition as well as in particle size. Both have their amino nitrogen bound in the form of an amide group, and both contain the "chromogenic material," yet there is a quantitative difference in the proportion of the partition. Although they may be considered as substances belonging to the same class of lipids, their occurrence in various organs also constitutes a point of difference. Normally, strandin occurs in less than 0.01% amounts in organs other than brain, as measured by the "chromogenic material" content of lipid extracts of organs, whereas the polycerebroside is present in about 10 times that amount in some spleens from patients with Gaucher's disease (Cases 1 and 2) and in about 5 times that amount in others (Cases 3 and 4). Since these values represent actual amounts of the purified polycerebroside isolated, it would be reasonable to assume that the true polycerebroside content of these spleens is much greater. Unfortunately, since the Gaucher polycerebroside differs so little in composition from cerebrosides in general, there is no way of determining it quantitatively by a specific analytical method (such as the neuraminic acid color reaction [16] for gangliosides), and estimates thus have to be based on the amount isolated under controlled conditions. Its migration as a homogeneous single boundary in the electrical field and its well-defined molecular weight, as determined by its diffusion coefficient and osmotic pressure, can be taken as attesting to a homogeneous entity, while the isolation procedure itself and the composition of the polycerebroside would tend to exclude consideration of any known contaminants. As pointed out previously, its acidic nature has to be ascribed to the presence of free carboxyl groups, in view of the absence of sulfur and the presence of phosphorus in only trace amounts. Solution of the problem of what residue contributes the free carboxyl groups would greatly facilitate envisagement of the chemical structure of the Gaucher polycerebroside. Klenk [16a] has satisfactorily explained the acidity of gangliosides on the assumption that the carboxyl group of neuraminic acid must be free and that the amino group is bound, because gangliosides do not have a free amino group prior to hydrolysis. Inasmuch as the Gaucher polycerebroside does not contain neuraminic acid, one would postulate either the presence of a hydroxycarboxylic acid bound in ether linkage through the hydroxyl group and having the carboxyl group free, or a higher analog of neuraminic acid (or amino polyglycol) soluble in chloroform and relatively insoluble in water. Conjectures along these lines obviously require further experimental data to be of any significance, and from the dearth of information on the actual structure of gangliosides and similar glycolipids one can easily envisage the magnitude of the challenge inherent in elucidation of the structure of as complex a lipid as the polycerebroside described.

When one considers the significance of the accumulation of polycerebrosides in organs in Gaucher's disease, particularly in the spleen, one problem presents itself with unusual insistence, resolving itself into consideration of the following questions with regard to the chemical pathogenesis of the disease: 1. Are polycerebrosides, like kerasin, normal constituents of tissue, and hence does their accumulation in Gaucher's disease represent the manifestation of a mechanism similar to

that suggested for kerasin?[11] 2. Is the polycerebroside-protoplasmic protein complex the precursor of the Gaucher lipoprotein, since partial hydrolysis in vitro of the polycerebroside does yield cerebrosides? 3. Does the polycerebroside represent a glycolipid totally foreign to normal tissue constituents—an abnormal product occurring in the course of the disturbed metabolism of lipoproteins in Gaucher's disease?

As emphasized previously in this discussion, the absence of an analytical method for their estimation in tissue renders the answer to the first question difficult, inasmuch as all estimates of the amount of polycerebroside present have to be based on the amounts isolated. Although I have failed to isolate the material from spleens of patients with other diseases (Banti's syndrome and hemolytic anemia), it nevertheless appears probable that traces of polycerebrosides do occur as constituents of normal tissue. Certainly, they can be recovered in small amounts from the white matter of normal brain (23 mg./100 gm. of fresh tissue). Hence, it might be argued reasonably that the accumulation of this water-soluble glycolipid in Gaucher's disease does follow the same type of process that leads to enrichment in the water-insoluble glycolipid kerasin in this disease.

Although an affirmative answer to the second question has certain attractions in providing an adequate and clear explanation for the eventual genesis of the Gaucher lipoprotein, any speculation on this point is yet far too premature. It becomes apparent that the answer to the third question depends largely on the answers that may eventually be formulated for the first two. Another explanation for the accumulation of this acidic polycerebroside in the Gaucher cell may well be sought in the high organic anion binding capacity of the Gaucher lipoprotein,[11] so that the accumulation of polycerebroside may represent the result of secondary binding by the Gaucher lipoprotein, as appears to be the case for the secondary binding of acidic phosphatides and the resulting enrichment of the phosphatide moiety of the total lipids in this disease. It may be pertinent to point out that the recovery of polycerebroside from the spleens of the older patients with Gaucher's disease (Cases 3 and 4) was much less than that from the younger ones (Cases 1 and 2), although all the spleens appeared equally heavily engorged with Gaucher cells on histologic examination. If, by future studies, this difference can be established to be a real one, the implication would undoubtedly be that the relationships between the Gaucher polycerebroside, occurring only in small amounts, and the Gaucher lipoprotein, accounting for the bulk of the kerasin "storage," may well consist in the transition or transformation of one into the other, with kerasin representing the "fixed" form ultimately present as the lipid moiety of the Gaucher lipoprotein. The rapid course, the more extensive involvement of organs, and especially the involvement of the central nervous system that characterize the infantile form of Gaucher's disease justify a closer examination of the polycerebrosides for their possible role in the institution of differences observed in the involvement of organs in the infantile form of Gaucher's disease, especially in so far as cerebral involvement is concerned. The close relationship the polycerebrosides bear to the gangliosides in composition and solubility serves to underscore their possible role in involvement of the central nervous system, particularly in view of the marked preponderance of gangliosides in the composition of lipids accumulating in the brains of patients with Tay-Sachs disease.

SUMMARY

The isolation of a new water-soluble glycolipid from the spleens of four patients with Gaucher's disease is described. Analytical data have shown this lipid to have long-chain saturated fatty acids, sphingosine or a sphingosine-like base, and one or more hexose residues as its constituents. This similarity in composition to cerebrosides, its large molecular size as determined by osmometry and diffusion studies (approximately 30,000), and the fact that a cerebroside similar to kerasin has been isolated from its partial hydrolysis products suggested the designation of "polycerebroside" for this new lipid which accumulates in the organs of patients with Gaucher's disease. Electrophoretic studies indicated that the lipid is polyacid in nature and migrates as a single boundary in the pH range from 4.5 to 8.6. The absence of neuraminic acid in its composition constitutes its main difference from the gangliosides described by Klenk. The relationship of the polycerebroside to the previously described lipoprotein of Gaucher's disease is discussed. In view of the known enrichment of nervous system cells in gangliosides that chemically characterizes the infantile type of Tay-Sachs disease, the possible role of the polycerebroside in the chemical pathogenesis of the central nervous system involvement observed in the infantile form of Gaucher's disease is suggested.

ESOPHAGEAL VARICES WITHOUT HEPATIC CIRRHOSIS

NORMAN GARRETT Jr., M.D.
AND
EDWARD A. GALL, M.D.
CINCINNATI

THE FREQUENT concomitance of cirrhosis of the liver and esophageal varices has been widely appreciated for over a century.[1] Indeed, the resulting concept of portal hypertension has become so deeply entrenched that standard texts uniformly relate varices in this location to some form of obstruction to the portal circulation.[2] The occurrence of pathologic venous dilatation in the absence of such a circumstance is given scant consideration.

In a series of 100 cases of Banti's syndrome, Thompson [3] stated that in all instances in which adequate studies were carried out, some form of obstructive process was found in relation to the portal venous system. Reporting 115 cases of hemorrhage from esophageal varices, Higgins [4] found that 80% of the patients had cirrhosis at necropsy or on the basis of clinical criteria. There was no amplification of the 20% in which the presence of cirrhosis was not established. On the other hand, Weinberg [5] found evidence of cirrhosis in only 20 of 95 patients with esophageal varices. Among the remainder, 51 had some form of heart disease, 5 revealed mediastinal masses secondary to bronchogenic carcinoma, and 19 suffered from a miscellany of disorders. The varices accompanying cirrhosis resulted in

This study has been supported in part by the Richard S. Austin Research Fund.

From the Department of Pathology, College of Medicine, University of Cincinnati, and the Cincinnati General Hospital.

1. Le Diberder and Fauvel: De l'hématémèse due à des varices de l'oesophage à propos de deux observations, Rec. trav. Soc. méd. obs. Paris **1**:257-284, 1857-1858. Power, W.: Enteritis: Varicose Veins of the Oesophagus, Maryland M. & S. J. **1**:316-318, 1839-1840.

2. Anderson, W. A. D., editor: Pathology, St. Louis, The C. V. Mosby Co., 1948. Boyd, W.: A Text-Book of Pathology: An Introduction to Medicine, Ed. 5, Philadelphia, Lea & Febiger, 1947. Cecil, R. L., and Loeb, R. F.: Textbook of Medicine, Ed. 8, Philadelphia, W. B. Saunders Company, 1951. Harrison, T. R.: Principles of Internal Medicine, Philadelphia, The Blakiston Company, 1950. Karsner, H. T.: Human Pathology, Ed. 7, Philadephia, J. B. Lippincott Company, 1949. Moore, R. A.: Textbook of Pathology, Ed. 2, Philadelphia, W. B. Saunders Company, 1951. Smith, L. W., and Gault, E. S.: Essentials of Pathology, Ed. 3, Philadelphia, The Blakiston Company, 1948.

3. Thompson, W. P.: The Pathogenesis of Banti's Disease, Ann. Int. Med. **14**:255-262, 1940.

4. Higgins, W. H.: The Esophageal Varix: Report of 115 Cases, Am. J. M. Sc. **214**:436-441, 1947.

5. Weinberg, T.: Observations on the Occurrence of Varices of the Esophagus in Routine Autopsy Material, Am. J. Clin. Path. **19**:554-557, 1949.

hemorrhage in 8 instances (40%) but there was bleeding in only one of the 75 noncirrhotic cases. It was remarked that the varicosities associated with cirrhosis were three times larger than those encountered in the other patients.

Weinberg's 95 cases were observed in a consecutive series of 1,189 autopsy examinations, an incidence of 8%. This contrasts very strikingly with the incidence found by Cleland,[6] who observed only two instances in 3,000 autopsies, and with our own experience of less than 1.5% in over 18,000 autopsies. Obviously, clearly stated minimal criteria are essential in order to avoid confusion of transitory congestive venous dilatation with true varices. The latter are undoubtedly the result of a relatively permanent alteration of vein structure, whatever the underlying cause. The former may well result from a postmortem dependent suffusion of blood or may actually represent misinterpretation of tangentially sectioned normal venules encountered on histologic examination.

Nonetheless, there seems little question that frank varices may occur in the absence of cirrhosis or other forms of portal obstruction. Nochimowski [7] collected 10 cases of this nature, the patients including three children. In each of these patients, ruptured varices had resulted in death from exsanguination. He cited the cases of two additional patients with idiopathic varicosities in which hemorrhage had not supervened. Duffy and Fraser [8] documented the unexpected death from bleeding varices of a 39-year-old soldier with dengue. The postmortem examination in this case revealed no cause for the varices. Friedman [9] reported death from severe hematemesis in a 3½-year-old boy with erosion of plexiform varices in the lower esophagus. The liver, spleen, and abdominal venous system in this child were all normal. Fischer [10] also cited the deaths from hemorrhage of three children with esophageal varices unrelated to portal or splenic disease. This author further commented upon the occasional appearance of varices in association with chronic esophagitis, with mediastinal compression due to goiter, and even as a manifestation of senescence. Several varices were encountered in a patient whose case was reported from the Massachusetts General Hospital,[11] a 72-year-old man who died of hypertension and acute myocardial infarction. In a recent report, Rack, Mincks, and Simeone [12] found 6 patients among 31 who had died with esophageal varices in whom organic liver disease was absent. In five of these patients there was chronic passive hyperemia of the liver, and in one the liver and spleen were normal.

6. Cleland, J. B.: Pathological Lesions Met with in the Oesophagus During Post Mortem Examination, M. J. Australia **1**:588-589, 1934.

7. Nochimowski, J.: Über tödliche Blutungen aus Oesophagusvarizen in Fällen ohne Lebercirrhose, Frankfurt. Ztschr. Path. **43**:463-475, 1932.

8. Duffy, D. G., and Fraser, A. N.: Fatal Bleeding from Oesophageal Varicosities, M. J. Australia, **1**:202, 1944.

9. Friedman, E.: Esophageal Varix: Report of a Case in a 3½-Year-Old Child, Not Dependent on Liver Cirrhosis, J. Pediat. **4**:641-647, 1934.

10. Fischer, W.: Störungen des Kreislaufs, in Henke, F., and Lubarsch, O., editors: Handbuch der speziellen pathologischen Anatomie und Histologie, Vol. 4: Verdauungsschlauch, Berlin, Springer-Verlag, 1926, Pt. 1, pp. 110-112.

11. Case Records of the Massachusetts General Hospital, Case No. 30292, New England J. Med. **231**:103-106, 1944.

12. Rack, F. J.; Mincks, J. R., and Simeone, F. A.: Observations on the Etiology of Esophageal Varices, A. M. A. Arch. Surg. **64**:422, 1952.

Kaplan [13] referred to two cases in which neoplastic compression of the superior vena cava resulted in the development of esophageal varicosities. In his paper the occurrence of varices in the newborn and the very young was noted. Instances of this nature have been considered to be congenital malformations.[14]

PRESENT STUDY

During a 26-year period (1926-1951) there were approximately 18,000 autopsies performed in the department of pathology at the Cincinnati General Hospital. The departmental diagnostic files list 257 instances of esophageal varices. For purposes of this study all cases were excluded in which review of the protocols or pertinent slides revealed evidence of hepatic cirrhosis or disease of the portal or splenic veins. Also deleted were those cases in which equivocal gross descriptions of varices were unsupported by study of microscopic slides.

There remained 13 cases (Table) in which esophageal varices were unaccompanied by significant liver cirrhosis. In several the possibility of portal vein obstruction could not be excluded with certainty. Obviously this survey does not represent a true index of the incidence of either esophageal varices in general or the idiopathic group in particular. Undue enthusiasm on the part of occasional prosectors resulted in the listing of varices in which justification for this designation probably did not exist. On the other hand, the remarkable collapse and loss of prominence of varicosities in the cadaver, coupled with a failure to prepare routine sections of the esophagus for microscopic examination, undoubtedly resulted in a modicum of error of omission.

As the files of our own department are perused and the literature investigated, it is apparent that there are no universally utilized minimum criteria for the recognition of esophageal varices. In our own group of cases, dilated or congested venules or tangentially sectioned veins were often unjustifiably classified as varicosities. In our selection of cases for this report we have considered the condition of the vein wall to represent a feature of paramount importance. We have required that the vessel be a vein with clearly developed intima, muscular media, and adventitia. The wall has exhibited irregular thinning, and there has been a variable degree of medial fibrosis with replacement of smooth muscle and elastica. In many there has been a mild nonspecific intramural and/or perivascular inflammatory reaction, and this has often been accompanied by adventitial red cell extravasation of greater or smaller amount. Although the vein diameter as measured on the microscopic slide represented an exceedingly variable feature, it was found that true varices rarely measured less than 3.0 mm. in a single diameter or had a cross sectional area of less than 2.0 sq. mm. (Figs. 2 and 3).

COMMENT

Pertinent data relating to each of the 13 cases are listed in the Table. There was no evidence of cirrhosis in the liver sections from 12 cases. One (Case 9) which had been described as nodular in gross description, revealed on section relatively slight portal fibrosis in an amount considered inadequate as a basis for portal

13. Kaplan, B.: Esophageal Varices, M. Rec. **154**:176-180, 1941.

14. Duffy and Fraser.[6] Kaplan.[13]

Esophageal Varices Without Hepatic Cirrhosis

Case	Age, Yr.	Race	Sex	Hematemesis	Ascites	Icterus	Heart Weight, Gm.	Heart Disease	Lung	Liver Weight, Gm.	Liver Disease	Spleen Weight, Gm.	Spleen Disease	Portal Vein	Esophageal Varices	Miscellaneous	Cause of Death
1	63	W	M	+	0	0	Normal	0	Aspirated blood	2,125	0	475	0	Normal	4 × 3 mm.	0	Exsanguination
2	60	W	M	+	0	0	Normal	0	Edema	2,450	Fatty	100	0	Normal	Massive	Acute gastric ulcer	Exsanguination
3	69	W	M	+	+	0	475	Mitral disease; acute myocarditis	Edema	1,300	0	400	Congestion	Normal	3.5 × 0.5 mm.	0	Heart failure
4	71	W	M	0	+	0	375	Myocardial infarct	Pneumonia; embolism	910	0	70	0	Normal	3.5 × 1.5 mm.	Cerebral infarct	Myocardial and cerebral infarcts
5	79	W	F	0	0	0	Enlarged	Hypertrophy; syphilitic aortitis	Pneumonia; congestion	875	Congestion (acute)	60	0	Normal	4 × 4 mm.	0	Heart failure
6	30	W	F	+ Not from varices	0	+	Normal	0	Pneumonia	1,550	0	125	0	Normal	Large	Superior mediastinal mass	Mediastinal Hodgkin's disease; generalized purpura
7	65	W	M	0	0	0	500	Myocardial infarct	0	1,360	0	150	0	Normal	Single, 7 × 3 mm.	0	Myocardial infarct
8	73	W	M	0	+	0	600	Rheumatic valvular disease	Pneumonia	1,650	Congestion (acute)	125	0	Normal	Single, 3 × 3 mm.	0	Heart failure
9	56	N	M	+	0	0	Normal	0	Edema	Normal	Slight portal fibrosis	584	Fibrosis	Dilated; portacaval shunt	5 × 3 mm.	0	Exsanguination
10	47	N	F	+	0	+	Normal	0	Pneumonia	1,700	Abscess; suppurative hepatitis	725	Septic	Normal	2.5 × 0.5 mm.	Hemolytic Streptococcus septicemia	Septicemia
11	80	W	M	0	0	0	Normal	0	Apical tuberculosis	5,200	Metastatic carcinoma	85	0	Intrahepat. ca. emboli	2 × 0.5 mm.	0	Carcinoma, stomach
12	72	W	M	0	0	+	Normal	0	Primary carcinoma	4,450	Metastatic carcinoma	150	Small metastases	Hilar mass	2 × 1 mm.	0	Carcinoma, lung
13	60	W	M	0	+ Bloody	+	Normal	0	Edema	3,620	Metastatic carcinoma	260	0	Hilar mass	2 × 1 mm.	Mesenteric artery thrombosis	Carcinoma, colon; gangrene, ileum

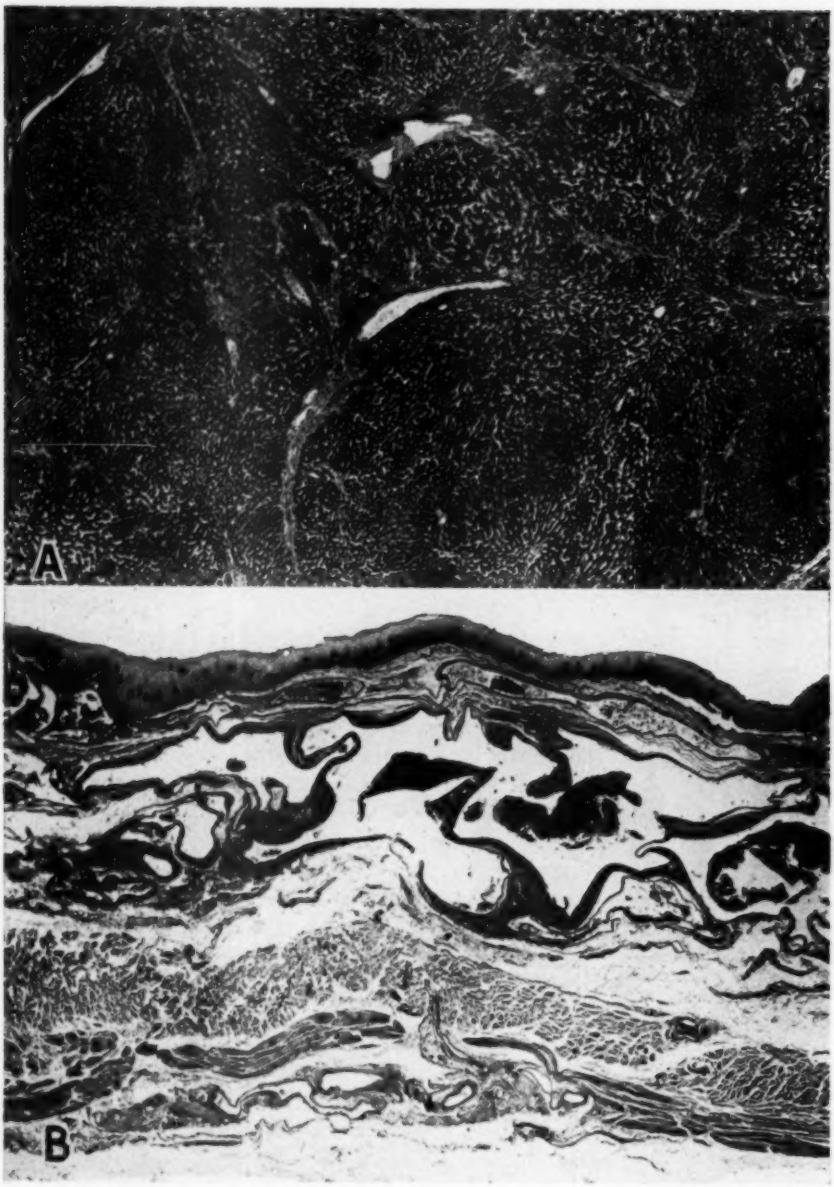


Fig. 1 (Case 9).—*A*, low power view of liver demonstrating spotty noncirrhotic portal area fibrosis; × 25. *B*, present in both mucosa and submucosa are plexiform varices, the walls of which are markedly thinned; × 12.

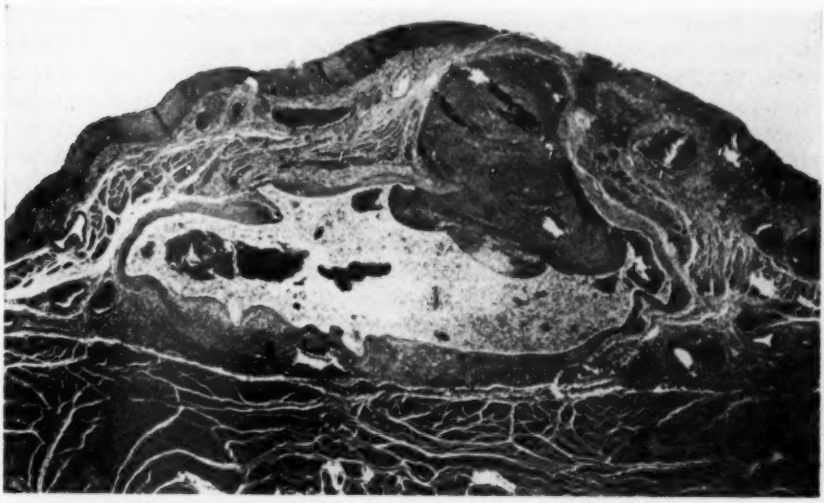


Fig. 2 (Case 1).—A large varix containing a thrombus. The vein wall abuts upon the mucosa, which has been ulcerated. The patient died as a result of hemorrhage from eroded lesions of this nature; × 16.

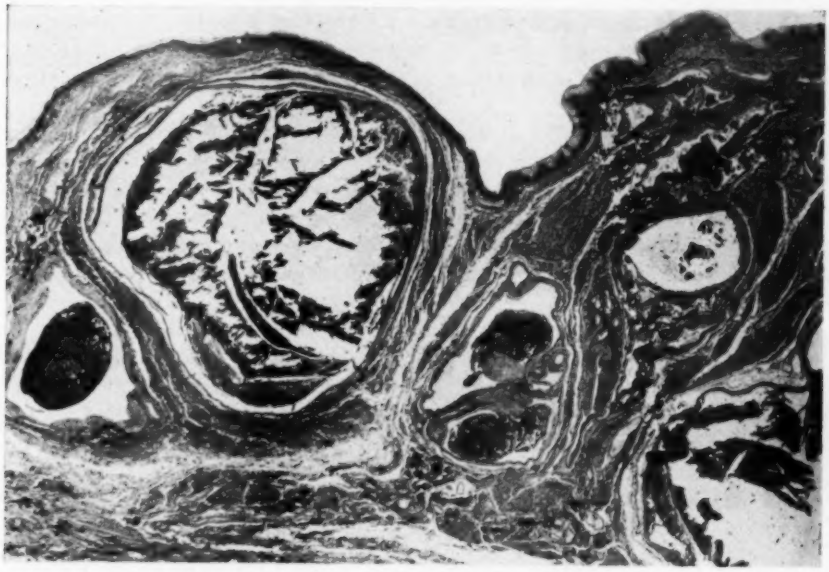


Fig. 3 (Case 5).—Several large varices producing nodular protrusion of the esophageal mucosa; × 12.

obstruction (Fig. 1*A*). However, in this case a large spleen had been removed at another hospital two years earlier and was said to have shown changes consistent with Banti's disease. Because of persistent varices and hematemesis the patient entered this hospital for a portacaval shunt and died of esophageal hemorrhage 10 days after operation (Fig. 1*B*). Necropsy revealed dilatation of the portal vein and its tributaries, for which no cause could be detected. In three other specimens, termed "cirrhosis" on gross examination (Cases 5, 7, and 10) because of "fine nodularity" or "tough texture," microscopic studies failed to support this presumption.

Five of the patients exhibited hematemesis of major proportions. This proved fatal in three (Cases 1, 2, and 9), but in one of these there was also hemorrhage from a complicating acute gastric ulcer. In two there was no disease of the liver (Cases 1 and 3), in one there was fatty infiltration only (Case 2) and in another a septic liver abscess (Case 10). A sixth patient (Case 6) with hematemesis did not bleed from the varices but suffered from a generalized purpura with multiple bleeding sites.

Among the eight patients in whom hemorrhage was not a factor, the liver was the seat of extensive metastatic carcinoma in three (Cases 11, 12, and 13) and severe passive congestion associated with heart failure in two (Cases 5 and 8). In three patients (Cases 4, 6, and 7) the liver was histologically normal. Two of these suffered myocardial infarctions which had only coincidental relationship to the varicosities. In Case 6 a large tumor mass filled the superior mediastinum and produced obstruction of the superior vena cava.

In two of the patients with metastatic carcinoma in the liver (Cases 12 and 13), hilar involvement was of such order that a presumption of portal vein compression seems justifiable, although evidence of thrombosis was lacking. In Case 11 there was microscopic evidence of neoplastic embolization of the minor intrahepatic portal radicles.

Thus in this series of 13 patients it seems reasonable to conclude that noncirrhotic liver disease or portal system stasis existed in five (Cases 9 to 13) and might conceivably have contributed to the development of the esophageal varices. In the remaining eight patients (Cases 1 to 8), no such circumstance existed. In one of these the condition may have been related to superior mediastinal obstruction, but in seven no cause for the occurrence of varicosities was detected.

SUMMARY

The occurrence of esophageal varices in the absence of cirrhosis or interference with the dynamics of the portal vein and its tributaries is uncommon. Few such cases are recorded in the literature. This report includes the data from 13 patients with varicosities of this nature. In three of these there was extensive neoplasm in the liver; in one there was a liver abscess, and in one the status of the spleen and splenic vein was in doubt. A sixth patient suffered from obstruction of the superior vena cava. Seven cases exhibited esophageal varices of idiopathic or primary character. Three of the patients died as the result of hemorrhage from the varices.

NATURE AND GENESIS OF PULMONARY ALTERATIONS IN CARBON TETRACHLORIDE POISONING

COMMANDER WILLIAM UMIKER (MC), U.S.N.
AND
JOHN PEARCE, M.D.
NEW YORK

DEATH IN carbon tetrachloride poisoning is usually the result of liver or kidney damage, and the pathological changes in these organs are well documented. The adrenal glands have also been found to be sensitive to this agent.[1] More recently attention has been directed towards myocardial changes.[2] However, descriptions of the changes in the lung in carbon tetrachloride poisoning have been scanty and the pathogenesis of the changes largely ignored.

In 1925, Gardner and his associates conducted extensive experiments on the effects of carbon tetrachloride on dogs.[1] The lungs of their animals showed hemorrhages, "brassy" edema, and patchy pneumonia. Four years later, MacMahon and Weiss [3] reported the case of a patient who died five days after drinking a carbon tetrachloride mixture. The lungs were voluminous, heavy, and edematous and microscopically revealed alveolar hemorrhages. The most remarkable feature was widespread fat embolism of the lungs and other viscera, a finding not reported by subsequent investigators. In 1935, Lehnherr [4] described opaque, bilateral, hilar, pulmonary shadows in the roentgenograms of a patient who had ingested carbon tetrachloride. These shadows slowly cleared as the patient recovered and had

---

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

This study was made possible through the courtesy of the Armed Forces Institute of Pathology. All but one of the case reports upon which it is based were obtained from the files of the institute.

From the Department of Pathology of the United States Naval Hospital, St. Albans, Long Island, N. Y., and the Departments of Surgery and Pathology of Cornell University Medical College and the New York Hospital.

1. Gardner, G. H., and others: Studies on the Pathological Histology of Experimental Carbon Tetrachloride Poisoning, Bull. Johns Hopkins Hosp. **36**:107, 1925.

2. Conaway, H. B., and Hoven, F.: Electrocardiographic Changes in Carbon Tetrachloride Poisoning: Report of a Case, U. S. Nav. M. Bull. **46**:593, 1946.

3. MacMahon, H. E., and Weiss, S.: Carbon Tetrachloride Poisoning with Macroscopic Fat in the Pulmonary Artery, Am. J. Path. **5**:623, 1929.

4. Lehnherr, E. R.: Acute Carbon Tetrachloride Poisoning: Report of a Case, Arch. Int. Med. **56**:98, 1935.

completely disappeared by the 50th day after ingestion. In 1946, Thompson [5] studied the roentgenographic changes in the lungs of 20 sailors who had been exposed to carbon tetrachloride fumes in a submarine. These changes varied from mild prominence of the pulmonary markings to complete lobar consolidation. One of the patients died, and at autopsy an acute hemorrhagic pneumonia was found. More recently, others have confirmed the occurrence of hemorrhagic pulmonary edema or hemorrhagic bronchopneumonia.[6]

TABLE 1.—*Ages, Survival Times, and Pulmonary Findings in Twenty-Seven Cases of Fatal Carbon Tetrachloride Poisoning*

Age	Survival Time (Days)	Ingestion	Inhalation	Weight of Lungs, Gm. (Combined)	Congestion	Edema	Hemorrhages	Bronchopneumonia	Fibrinous Exudate	Pseudomembrane	Alveolar Lining Cell Proliferation	Fibroblastic Proliferation
31	1	?	?	1,850	+	?	+	..	..	..	..	..
21	3	+	..	?	+	..	..	..	..	..	..	..
45	3	+	..	1,360	+	+	+	+	..	..	..	..
22	4	+	..	?	+	+	+	+	..	..	..	..
35	5	+	..	1,830	+	+	+	+	..	..	..	..
33	7	+	..	?	+	+	+	..	..	..	..	..
33	8	?	?	1,055	+	+	+	..	..	..	..	..
31	9	..	+	?	+	+	+	+	+	+	+	..
31	10	+	..	?	+	+	+	..	+	+	+	+
32	10	..	+	1,380	+	+	+	..	..	..	..	..
26	10	+	..	2,700	+	+	+	+	..	+	+	..
22	10	+	..	1,500	+	+	+	+	+	+	+	+
37	10	..	+	1,850	+	+	+	..	+	+	..	..
40	10	..	+	3,500	+	+	+	+	..	..	+	+
27	11	..	+	2,100	+	+	+	..	+	+	+	..
24	11	..	+	?	+	+	+	+	..	+	..	..
26	11	..	+	1,570	+	?	+	..	..	+	+	+
29	12	+	..	1,225	+	+	+	+	..	..	..	..
35	14	..	+	2,400	+	+	+	..	..	..	+	..
29	14	+	..	2,180	+	+	+	+	..	+	+	+
39	14	+	..	?	+	+	+	..	..	+	..	+
31	15	..	+	2,620	+	+	+	+	+	+	+	+
28	15	+	..	2,560	+	+	+	..	+	+	+	..
27	15	..	+	?	+	+	+	+	+	+	+	+
25	16	..	+	?	+	+	+	..	..	+	+	..
37	?	..	+	2,300	+	+	+	+	+	+	+	..
26	16	..	+	2,960	+	+	+	..	+	+	+	+

As indicated by the articles cited and by our recent experience with a fatal case of carbon tetrachloride poisoning, there is no doubt that pulmonary alteration accompanies toxic exposure to this chemical. It is the aim of this paper to describe the lung lesions and to discuss their genesis.

For this purpose the autopsy protocols and microscopic sections from 26 cases of fatal carbon tetrachloride poisoning on file at the Armed Forces Institute of Pathology, together with 1 from the St. Albans Naval Hospital, were studied. The general clinical and the roentgenographic and pathological features as they apply to the lung are described hereafter.

5. Thompson, C. M.: Pulmonary Changes in Carbon Tetrachloride Poisoning, Am. J. Roentgenol. **55**:16, 1946.

6. Foxell, A. W. H.: Three Cases of Carbon Tetrachloride Poisoning with One Fatality, Brit. M. J. **1**:397, 1951. Prag, J. J.: Carbon Tetrachloride Poisoning by Inhalation, South African M. J. **25**:351, 1951.

CLINICAL

The patients were all men aged between 21 and 45 years. A history of chronic alcoholism was frequently elicited, and many of the patients first became ill after a bout of acute alcoholism. Their courses were characterized by clinical and laboratory evidence of liver and kidney failure. The intervals between time of exposure and time of death varied from 1 to 16 days, with the greatest number of deaths occurring on the 10th day. Hepatic damage predominated in patients who died within the first week, while renal insufficiency with uremia was the most important feature in patients who died later. However, it was frequently difficult to determine which was the major factor.

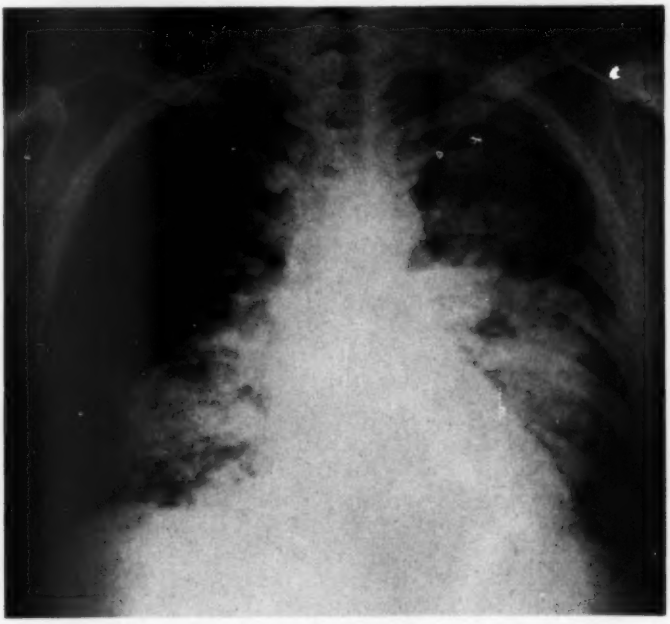


Fig. 1.—Roentgenogram taken nine days after exposure to carbon tetrachloride fumes, showing fluffy hilar shadows. (A. F. I. P. Acc. No. 168546, photograph No. 95268.)

Twelve men had been poisoned by ingestion of the chemical and thirteen by inhalation of its fumes. In two instances the type of exposure could not be ascertained. Table 1 lists the patients' ages, the type of exposure, and the intervals between exposure and death. Although the patients who had ingested carbon tetrachloride tended to have a more fulminating course, not all of them did so. The average survival time of the ingestion group was 9 days and that of the inhalation group 12 days. It should be noted that five of the former group died within the first week, whereas the first death in the latter group did not occur until the ninth postexposure day.

Respiratory symptoms and signs were usually not striking features until the latter portion of the patients' courses. Dyspnea was often an early symptom and

frequently was accompanied by pulmonary rales. Dyspnea and cyanosis became prominent during the terminal days. In a few instances the attending physician noted that the respiratory symptoms seemed to be out of proportion to the severity of the hepatic and renal damage. Occasionally there were physical signs of consolidation.

ROENTGENOGRAPHIC EXAMINATION

Results of roentgenographic examinations were recorded in 12 cases. Abnormalities were found in all but one of these. The earliest change appeared on the ninth day after exposure and the incidence of positive findings increased each additional day thereafter.

Fig. 2.—Hemorrhagic consolidation of lung, producing a spleen-like appearance. Patient died 16 days after exposure to carbon tetrachloride fumes.

The changes consisted of fluffy, hilar opacities of varying densities, which on subsequent examinations gradually extended peripherally (Fig. 1). In no instance did the lesion extend quite to the pleural surface. Widening of the cardiac silhouette was frequently noted, but it could not be ascertained with certainty whether the pulmonary changes preceded or followed the cardiac enlargement.

PATHOLOGY

Gross.—In almost all cases, the lungs were described as being voluminous, dark-red, and heavy (Fig. 2). Bloody edema fluid exuded spontaneously from the cut surfaces or could be readily expressed. Irregular, poorly defined areas of

consolidation of various sizes were usually noted. These were dark red-purple, friable, or fleshy masses which on the cut surface bulged from the adjacent parenchyma. Other terms describing the gross characteristics were "liver-like," "spleen-like," "jelly-like," and "meaty." Most prosectors interpreted these abnormalities as representing hemorrhagic pneumonia or hemorrhagic edema. In addition, many of the lungs presented the typical grayish consolidations of purulent bronchopneumonia. The gross findings are summarized in Table 1.

The tracheobronchial tree was usually unremarkable except for congestion. Small intrapleural hemorrhages were often present, but increased pleural fluid was seldom noted.

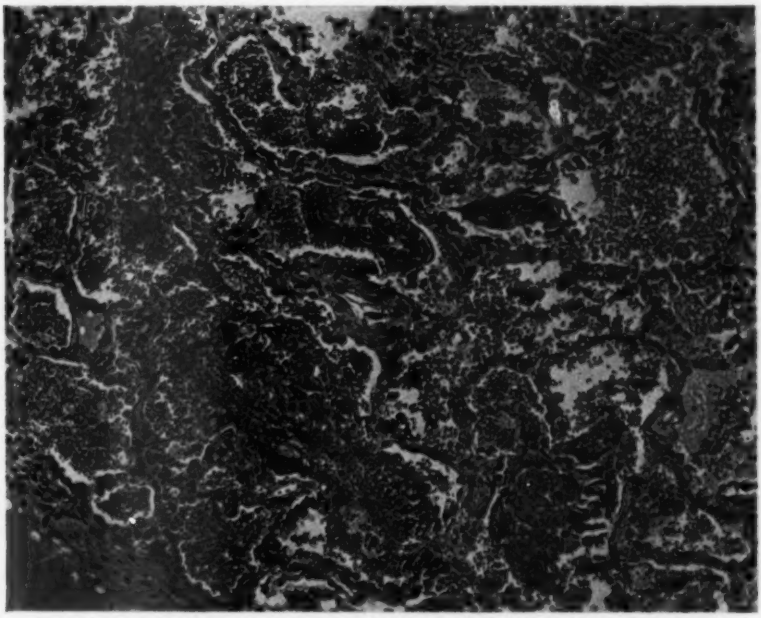


Fig. 3.—Microscopic appearance of the lungs shown in Figure 2, with acute passive congestion, intrapulmonary hemorrhage, and thickening of alveolar walls (× 150).

As this investigation was limited to the pulmonary changes, no detailed study of the cardiac abnormalities was made. However, right-sided cardiac dilatation was frequently noted, whereas dilatation of the chambers on the left side was rarely mentioned.

Microscopic.—Intense acute passive congestion and hemorrhagic edema were constant findings (Fig. 3). About one-half the patients had purulent bronchopneumonia, which was encountered as frequently in the men who died a few days after exposure as in those who lived longer. The purulent pneumonia was relatively minimal in most instances but occasionally was sufficiently severe to be regarded as the immediate cause of death.

In contrast to the changes which showed no correlation with survival time and are regarded as incidental findings, there were abnormalities directly related to the

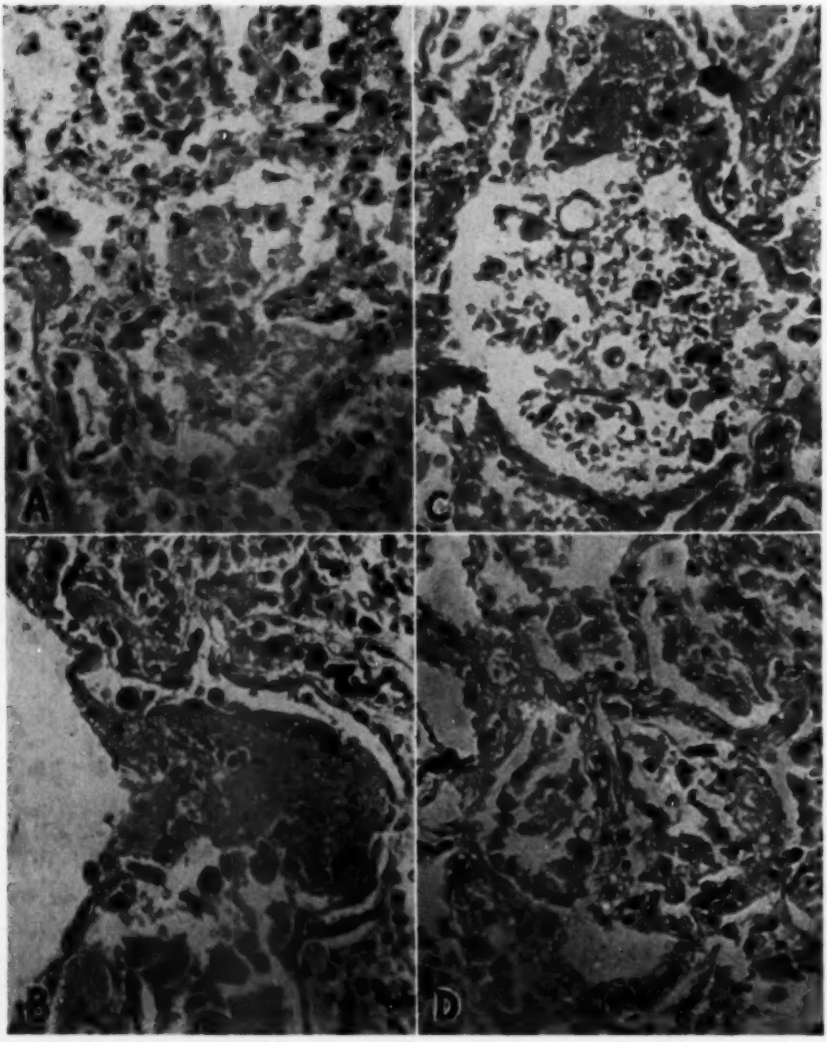


Fig. 4.—*A*, carbon tetrachloride poisoning: fibrin and scattered leucocytes in alveolar lumens, minimal thickening of alveolar walls. (A. F. I. P. Acc. No. 239887; × 220.) *B*, carbon tetrachloride poisoning; alveolar duct (on left) with adjacent alveolus filled with fibrinous exudate; prominent lining cells; hemosiderin-filled macrophages in lower portion of photograph (× 220). *C*, similar changes in lung of patient who died in uremia secondary to lower nephron nephrosis after incompatible-blood transfusion (× 220). *D*, same case: marked proliferation of alveolar lining cells (× 220).

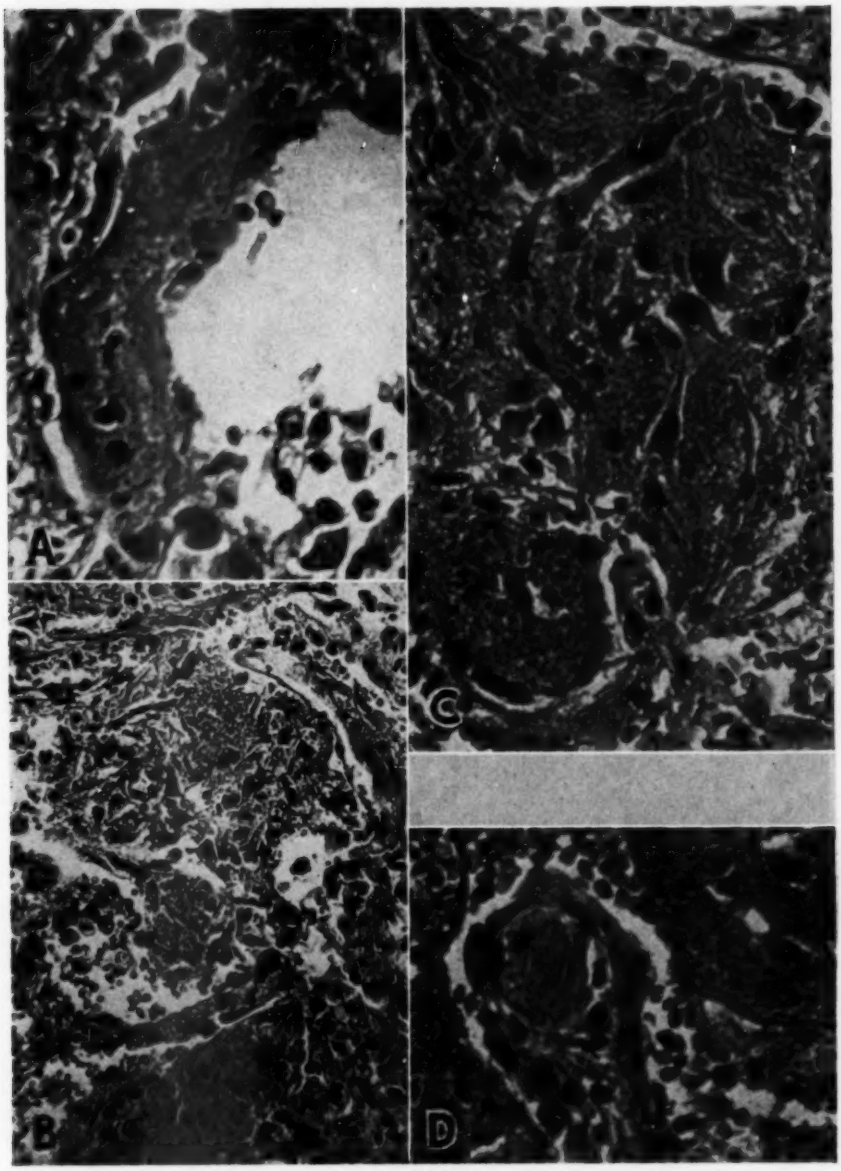


Fig. 5.—Carbon tetrachloride poisoning. *A*, alveolus partially lined by thick pseudomembrane of acidophilic material (× 450). *B*, organization of alveolar exudate (× 220). *C*, higher magnification of same area, showing proliferating fibroblasts and angioblasts (× 450). *D*, thrombus within dilated vessel (× 450).

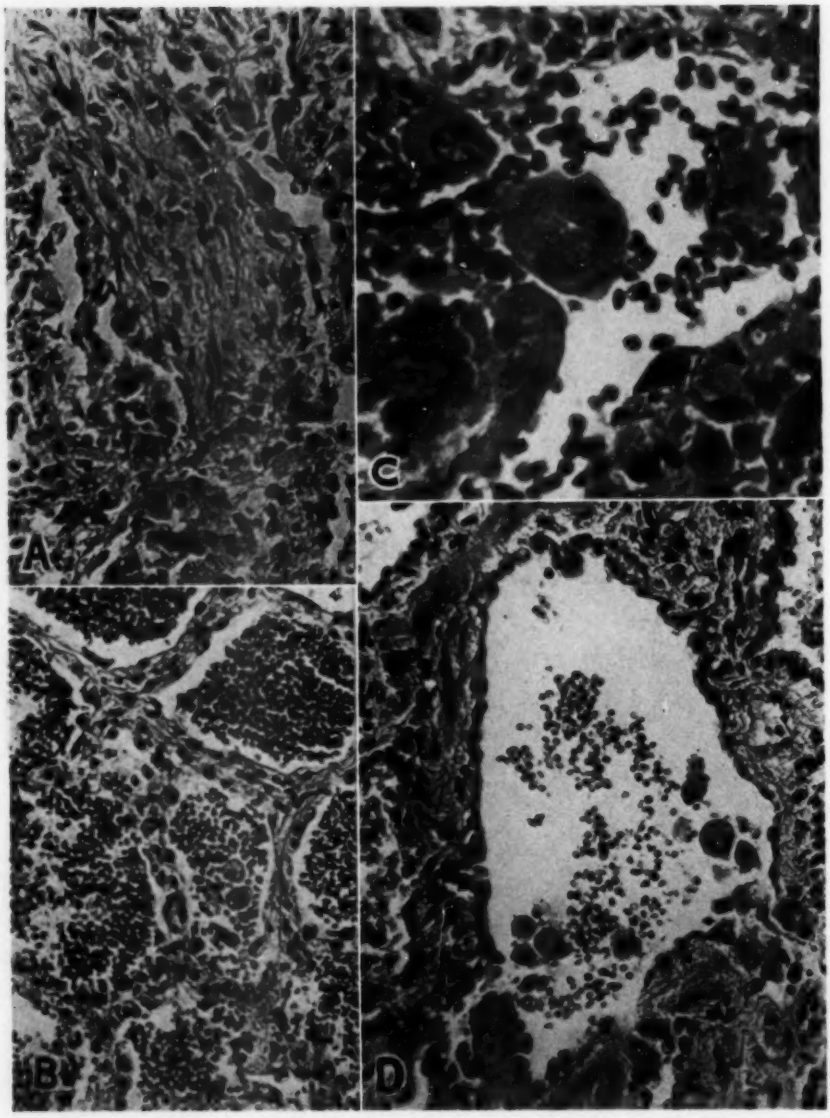


Fig. 6.—Carbon tetrachloride poisoning. *A*, edema and fibroblastic proliferation of interalveolar septum (× 200). *B*, thickened alveolar walls, with prominent lining cells and recent intraluminal hemorrhages (× 150). *C*, bizarre alveolar lining cells (× 410). *D*, replacement of normal bronchiolar epithelium by a single layer of cuboidal cells (× 200). Note the prominent alveolar lining cells in adjacent alveoli.

period of survival. These alterations appeared first on the ninth day after exposure, thus paralleling in time of appearance the roentgenographic changes, and they increased in intensity with survival time. They were present in 18 of the 20 patients who lived longer than eight days. They consisted of fibrinous exudate, thickening of alveolar walls by proliferating fibroblasts, and finally, proliferation of cells resembling epithelium and lining the alveoli.

Fibrin was found within the alveolar lumens and to a lesser degree within the alveolar walls (Fig. 4, *A* and *B*). Frequently this material had congealed into eosinophilic masses in which macrophages and rare polymorphonuclear leucocytes were embedded. The exudate either remained in the central portions of the alveolar lumens or formed a coating of the inner alveolar walls, thus producing a striking resemblance to the so-called vernix membrane seen in neonatal deaths (Fig. 5*A*). It was sometimes difficult to determine whether this pseudomembrane was lining or replacing the alveolar walls. It could be traced from terminal bronchioles and alveolar ducts into alveoli. The intraluminal deposits were often organized (Fig. 5, *B* and *C*). Lymphocytes, mononuclear phagocytes, and numerous polymorphonuclear leucocytes were scattered through the alveolar walls, in the interlobular septa, and to a much lesser extent within the alveolar lumens. A few small vessels were occluded by fibrin thrombi (Fig. 5*D*).

Proliferating fibroblasts were numerous within the alveolar walls and the larger pulmonary septa. The latter were edematous and greatly widened (Fig. 6*A*). Hyperplasia and hypertrophy of alveolar lining cells were striking features, especially in those patients who had survived two or more weeks (Figs. 2, 3, 4, and 5). These cells frequently contained large, bizarre, and occasionally multiple nuclei (Fig. 6*C*). Macrophages, most of which contained hemosiderin pigment, were numtrous, especially in regions of hemorrhage. The combination of congestion, fibroblastic proliferation, pseudomembrane formation, and alveolar lining cell hyperplasia resulted in a marked thickening of the alveolar walls (Fig. 6*B*).

The larger bronchi were unremarkable, but the smaller branches and the alveolar ducts were affected. Their mucosal linings were partially or completely desquamated, with only bare vascular stroma or a lining of a single layer of low cuboidal cells remaining (Fig. 6*D*).

Sections from the peripheral zones of the lungs differed from sections from the hilar zones only in revealing patchy emphysema.

COMMENT

Since 18 of the 20 patients who lived longer than eight days after their fatal exposure to carbon tetrachloride had pulmonary lesions that not only were severe but also were strikingly similar, it is reasonable to conclude that these lesions resulted from the poisoning. The mechanism of their production is not obvious. The characteristic change occurs only after eight days. The presence of edema, congestion, hemorrhage, and bronchopneumonia does not correlate with duration of survival or route of entry of the toxins, and therefore these components of the lesion are probably incidental and the result of shock, infection, and factors other than the carbon tetrachloride.

Thompson in his discussion of the lung alterations repudiated the previously held belief that pulmonary damage occurred only when carbon tetrachloride was

decomposed into phosgene gas by heat. He expressed the opinion that the changes were due to the direct action of carbon tetrachloride fumes per se.

That the damage is not caused by direct action of inhaled carbon tetrachloride fumes on the lungs is attested by the fact that it was equally severe in persons who were poisoned by drinking the fluid and was completely absent in one patient who inhaled the gas. The lack of inflammation in the trachea and larger bronchi is also inconsistent with a direct irritant action, and in addition the histological findings are unlike those produced by poison gases.[7]

Although it has been shown that carbon tetrachloride is excreted chiefly through the lungs,[8] such a secondary exposure is a poor explanation, both because the rate of excretion is greatest immediately after ingestion and because the inhalation victim's lungs would have a double exposure and therefore should have a more severe reaction. In either circumstance, direct irritant action or secondary contact during excretion, it would be expected that the greatest anatomical change would occur shortly after intake of the chemical rather than nine or more days later.

TABLE 2.—*Ages, Survival Times, and Pulmonary Findings in Nine Cases of Uremia*

Age	Survival Time, Days	Weight of Lungs (Combined), Gm.	Congestion	Edema	Hemorrhages	Bronchopneumonia	Fibrinous Exudate	Pseudomembrane	Alveolar Lining Cell Proliferation	Fibroblastic Proliferation
19	14	1,050	+	+	+	+	+	..	+	+
39	11	1,670	+	+	+	+	+	+	+	+
28	5	1,450	+	+	+	..	+	+	+	..
?	10	980	+	+	+	+	+	..	+	..
48	6	1,140	+	?	?	+	..	..	..	..
43	11	2,490	+	+	+	+	+	+	+	..
22	?	1,000	+	+	+	..	..	..	..	..
34	5	2,300	+	+	+	+	+	+	+	+
28	9	700	+	+	+	+	+	..	+	+

In the case reported by MacMahon and Weiss there was widespread fat embolization of the lungs and other viscera. These authors suggest that pulmonary change is secondary to this embolization and propose the necrotic liver as a source of the fat. In none of the 27 cases studied by us was there any indication of fat emboli.

Early in this study it became evident that there was a close similarity between the lung changes in carbon tetrachloride poisoning and those found in miscellaneous uremic states. Attempts were therefore made to compare the changes in a series of patients with uremia of different causes with those due to carbon tetrachloride poisoning. Unselected uremic patients proved to be unsatisfactory for this purpose because most of them had associated conditions, such as chronic passive congestion secondary to cardiac failure, or were aged persons with severe emphysema, bronchopneumonia, or atelectasis. These associated conditions made it difficult to evaluate properly the changes secondary to the uremia. Therefore a smaller but more satis-

7. Durlacher, S. H., and Bunting, H.: Pulmonary Changes Following Exposure to Phosgene, Am. J. Path. **23**:679, 1947.

8. Lehman, K. B., and Flury, F.: Toxicology and Hygiene of Industrial Solvents, Baltimore, Williams & Wilkins Company, 1943, p. 145.

factory group was selected, consisting of nine patients who had severe and rapidly developing azotemia. Five had renal failure following whole blood transfusion, surgery, or both. Two patients had acute fulminating glomerulonephritis; one died of arsenical poisoning and one of periarteritis nodosa.

The major microscopic abnormalities in this group are listed in Table 2. Essentially the changes were similar to those in the carbon tetrachloride group, the only differences being in degree (Fig. 4, *C* and *D*). Although the acidophilic exudate in the central portions of the lumens or lining the alveolar walls was generally less in amount in the uremic group, it was a constant finding. The characteristic thickening of the alveolar walls by a combination of congestion, fibrosis, and proliferation of alveolar lining cells was striking in most instances. There were edema, cellular infiltrations, and fibroblastic proliferation within the pulmonary septa, and scattered inflammatory cells, chiefly neutrophilic polymorphonuclear leucocytes, infiltrated the alveolar walls. Fibrin thrombi were occasionally encountered in small blood vessels. Macrophages, usually containing hemosiderin, were numerous.

The lesions in these nine uremic patients conformed in general with those described by Bass and Singer [9] as a result of their investigations of the lung in uremia. They refer to the main feature as a "fibrinous alveolitis." The lungs of the patients poisoned by carbon tetrachloride revealed changes which differed only by their greater intensity. The accompanying severe liver damage, with resultant defects in blood coagulation and serum protein formation, may explain the greater outpouring of fluid and cells into the pulmonary parenchyma.

The x-ray appearances of the lungs of the patients with carbon tetrachloride poisoning were characterized by hilar, fluffy opacities which extended peripherally as the disease progressed. These changes are identical with those reported in uremic patients.[10] In the patients with carbon tetrachloride poisoning neither the anatomical nor the roentgenographic alterations occurred before the ninth day, at which time azotemia was well established.

The similarities described strongly suggest that the anatomical alterations in the lungs of persons poisoned by carbon tetrachloride are a part of the uremic state caused by the kidney necrosis rather than a direct effect of the chemical. It is not the purpose of this paper to enter into the problem of how retention of metabolites can give rise to a pneumonitis, but the inflammatory character of the "fibrinous alveolitis" has a parallel in the fibrinous pericarditis that precedes death in uremia.

SUMMARY

The pulmonary changes in 27 young men who died of carbon tetrachloride poisoning have been described. The basic lesion consisted of an exudate of fibrin, a pseudomembrane lining the alveolar walls, a thickening of alveolar walls by growing fibroblasts, and a proliferation of cells resembling epithelium lining the alveolar walls.

9. Bass, H. E., and Singer, E.: Pulmonary Changes in Uremia, J. A. M. A. **144**:819, 1950.

10. Roubier, C., and Plaucku, M.: Sur certains aspects radiographiques de l'œdème pulmonaire chez les cardio-rénaux azotémiques, Arch. méd.-chir. app. respir. **9**:189, 1934. Rendich, R. A.; Levy, A. H., and Cove, A. M.: Pulmonary Manifestations of Azotemia, Am. J. Roentgenol. **46**:802, 1941. Bass.[9]

The appearance of the lungs in nine cases of rapidly developing uremia in patients of comparable ages whose renal failure had various causes has been compared with that in the carbon tetrachloride group and found to be essentially the same.

The roentgenograms of the lungs of the patients with carbon tetrachloride poisoning are similar to those that have been described for uremic patients.

It is highly probable that the pulmonary changes in carbon tetrachloride poisoning are the result of the uremia produced by the necrotizing action of that chemical on the kidney rather than the result of its direct action on the pulmonary parenchyma during either inhalation or excretion.

Case 1 (A. F. I. P. No. 269373).—A white man aged 31 was found dead in bed with a cup of carbon tetrachloride at the bedside. Carbon tetrachloride was isolated from the brain but not from the gastric contents. Autopsy revealed acute congestion of the viscera. The lungs weighed 1,850 gm. and showed hemorrhagic edema; the cut surface was mottled reddish-black. Microscopic examination disclosed marked congestion and slight edema, focal atelectasis, postmortem autolysis, and diffuse intrapulmonary hemorrhages.

Case 2 (A. F. I. P. No. 104604).—A white man aged 21 drank an unknown quantity of carbon tetrachloride kept in a whisky bottle. Delirium, shock, abdominal pain, emesis, and uremia developed. Marked cyanosis appeared three hours before death. The patient died 58 hours after ingestion of the fluid. Autopsy revealed generalized congestion of the viscera. The lungs showed marked acute passive congestion.

Case 3 (A. F. I. P. No. 208128).—A white man aged 45, with acute and chronic alcoholism, had been drinking vanilla extract and other fluids for several weeks (over a two-day period prior to death). He received 12 cc. of paraldehyde orally; this was later found to contain 40% carbon tetrachloride. Coma developed. An x-ray taken with a portable machine was unrevealing. Autopsy disclosed acute hepatitis and nephritis. The lungs weighed 1,390 gm. and were intensely congested. Microscopic examination revealed marked congestion and moderate edema, fibrinopurulent bronchopneumonia, edema of the septa, and recent focal hemorrhages.

Case 4 (A. F. I. P. No. 79867).—A Negro aged 22, admitted for treatment of hookworm infestation, received four 1.33-cc. capsules of carbon tetrachloride. He became restless, with nausea and emesis, and then became comatose and cyanotic. Progressive respiratory distress developed, with death four days after ingestion of the capsules. Autopsy disclosed acute yellow atrophy. The lungs showed marked congestion and edema. Microscopic examination revealed marked congestion and edema, focal atelectasis, early purulent bronchopneumonia, focal hemorrhages, and clusters of pigmented macrophages.

Case 5 (A. F. I. P. No. 150578).—A white man aged 35, a chronic alcoholic, drank about 15 cc. of carbon tetrachloride accidentally. Jaundice and edema (peripheral) developed. Cyanosis appeared on the day of death, five days after ingestion of the fluid. Autopsy disclosed acute hepatitis and nephritis. The lungs weighed 1,830 gm. There was marked edema. The cut surface was mottled red, suggesting red hepatization. Microscopic examination revealed marked congestion and edema, severe fibrinopurulent bronchopneumonia, and focal pulmonary hemorrhages.

Case 6 (A. F. I. P. No. 97414).—A white man aged 33, a chronic alcoholic, accidentally ingested carbon tetrachloride at an undetermined time. There was marked cyanosis at the time of admission. He died seven days later. Autopsy disclosed acute hepatitis and nephritis. The lungs showed congestion and edema. Microscopic examination revealed marked congestion and edema, focal hemorrhages, aspirated gastric contents with digestion of pulmonary parenchyma, and numerous pigmented macrophages.

Case 7 (A.F.I.P. No. 164214).—A white man aged 33, a chronic alcoholic, had no history of carbon tetrachloride exposure. He died eight days after admission. Toxicological studies of the kidney and liver gave positive results for carbon tetrachloride. Autopsy disclosed acute hepatitis and nephritis. The lungs weighed 1,055 gm. and showed congestion and edema. There were less well aerated, reddish-purple, slightly rubbery subpleural areas. Microscopic examination revealed moderate congestion, aspirated gastric contents, and rare hemorrhages.

CASE 8 (A. F. I. P. No. 71632).—A white man aged 31 was employed in an airplane hangar and liked to smell carbon tetrachloride fumes. He had a bout of acute alcoholism just prior to admission. There were symptoms of gastroenteritis, with dyspnea, and cyanosis on the day prior to death. An x-ray showed an area of increased density in the right middle lobe. He died nine days after admission. Autopsy revealed acute hepatitis and nephritis and bronchopneumonia. There was consolidation of the right upper lobe. Microscopic examination disclosed marked congestion and edema, focal atelectasis, focal hemorrhages, purulent bronchopneumonia, fibrin within alveoli, hyaline membrane, and slight alveolar lining cell hyperplasia.

CASE 9 (A. F. I. P. No. 42824).—A white man aged 31, a chronic alcoholic, drank 3 oz. of carbon tetrachloride accidentally. X-ray examination of the lungs showed increased hilar markings. There was deep cyanosis on the day prior to death, 10 days after ingestion of the fluid. Autopsy disclosed acute nephritis and hepatitis. The lungs were edematous. Microscopic examination revealed marked congestion and moderate edema; focal hemorrhages; fibrin within alveoli, some with organization; moderate proliferation of alveolar lining cells; thickened alveolar walls; hyaline membrane; fibrinoblastic reaction, and numerous pigmented and nonpigmented macrophages.

CASE 10 (A. F. I. P. No. 69873).—A white man aged 32, with moderate alcoholism, cleaned machine gun parts with carbon tetrachloride and died 10 days later of uremia. Autopsy disclosed acute hepatitis and nephritis. The lungs weighed 1,380 gm. and showed marked congestion and edema. Microscopic examination revealed marked congestion and moderate edema, focal atelectasis, focal hemorrhages, and foci of pigmented macrophages.

CASE 11 (A. F. I. P. No. 136441).—A white man aged 26 drank a mouthful of carbon tetrachloride from a gin bottle. Cough, dyspnea, cyanosis, and hemoptysis had developed at least one day prior to death. X-rays showed an inflammatory process in the lower portions of several lobes. He died 10 days after ingestion of the fluid. Autopsy disclosed acute hepatitis and nephritis. The lungs weighed 2,790 gm. and showed pleural hemorrhages, hemorrhagic edema, focal hemorrhages, and a fleshy consistency, with some areas which on the cut surface were more elevated and firmer. Microscopic examination revealed marked congestion and edema, purulent bronchopneumonia, focal hemorrhages, slight-to-moderate alveolar wall thickening, hyaline membrane formation, and alveolar lining cell proliferation.

CASE 12 (A. F. I. P. No. 147021).—A white man aged 22 accidentally drank a "slug" of carbon tetrachloride from a whisky bottle. Nausea, emesis, and abdominal pain developed. He died in coma 10 days after ingestion of the fluid. Autopsy disclosed acute nephritis. The lungs, which weighed 1,500 gm., showed pleural hemorrhages; poorly demarcated, firm areas with dark-red, smooth, glassy cut surfaces, and focal hemorrhages. Microscopic examination revealed moderate congestion and edema; focal hemorrhages; early purulent bronchopneumonia; marked thickening of alveolar walls; marked proliferation of alveolar lining cells; hyaline membrane; fibrin within alveoli, some with organization; foci of fibroblastic reaction, and numerous macrophages, with or without pigment, within alveoli.

CASE 13 (A. F. I. P. No. 263239).—A white man aged 37 cleaned refrigerator parts with carbon tetrachloride on two consecutive days and died 10 days after the first exposure. Autopsy disclosed acute nephritis and hepatitis. The lungs weighed 1,850 gm. They were heavy and soggy. The cut surface was beefy-red, nongranular, and fleshy. There was hemorrhagic edema. Microscopic examination revealed marked congestion; moderate edema; intrapulmonary hemorrhages; fibrin, some organizing, within alveoli; hyaline membrane (slight), and numerous pigmented and nonpigmented macrophages in alveoli.

CASE 14 (A. F. I. P. No. 168546).—A white man aged 40 fell asleep in bed in a hotel while smoking. The mattress caught fire, and the fire was extinguished by a bellhop with carbon tetrachloride apparatus. The patient was admitted two days later with cough. An x-ray three days prior to death showed increased central lung markings. Two days later diffuse clouding suggested pulmonary edema. The patient died 10 days after exposure. Autopsy disclosed mild hepatitis and nephritis. The lungs weighed 3,500 gm., they were voluminous, heavy, and sodden. There were pleural hemorrhages and hemorrhagic edema. Microscopic examination revealed moderate congestion and severe edema, fibrinopurulent bronchopneumonia, marked thickening of alveolar walls, fibroblastic proliferation in alveolar walls, and proliferation of alveolar lining cells, many of the cells having giant nuclei.

Case 15 (A. F. I. P. No. 116785).—A white man aged 27 used carbon tetrachloride as a paint remover in an engine room. An admission x-ray was normal. An x-ray taken on the day prior to death showed bilateral infiltrations. There was marked cyanosis on the day of death, which occurred 5 days after admission, 11 days after the first exposure. Autopsy disclosed acute hepatitis and nephritis. The lungs weighed 2,100 gm. They were mottled, with diffuse hemorrhagic discoloration, congestion, and edema. Microscopic examination revealed marked congestion and edema; focal hemorrhages; some alveolar lining cell hyperplasia and hypertrophy; fibrin within alveoli, some with organization; hyaline membrane, and numerous macrophages.

Case 16 (A. F. I. P. No. 119342).—A white man aged 24 used carbon tetrachloride aboard a submarine as a grease solvent. There developed nausea, emesis, weakness, cough, hemoptysis, cyanosis, and increasing respiratory difficulty. He died 11 days after exposure. Autopsy disclosed toxic hepatitis and nephritis. The lungs were heavy, congested, edematous, and largely consolidated. There were pleural hemorrhages. Microscopic examination revealed marked congestion and edema, atelectasis, purulent bronchopneumonia, early hyaline membrane formation, minimal focal hemorrhages, and large numbers of pigmented macrophages.

Case 17 (A. F. I. P. No. 236150).—A Negro aged 26 cleaned rugs with carbon tetrachloride. Cough, dyspnea, hemoptysis, and cyanosis developed four days after exposure. An x-ray showed "pneumonia" or accentuation of vascular markings bilaterally, especially in the right subhilar region. He died 11 days after exposure, of uremia. Autopsy disclosed acute nephritis and hepatitis. The lungs, weighing 1,570 gm., had a liver-like consistency, were mottled grayish-brown, and showed hemorrhagic edema. Microscopic examination revealed severe congestion, slight edema, focal atelectasis, slight focal hemorrhages, hyaline membrane, thickened alveolar walls, moderate proliferation of alveolar lining cells, some fibroblastic reaction within alveolar walls, and numerous pigmented macrophages.

Case 18 (A. F. I. P. No. 168427).—A white man aged 39, with chronic alcoholism, accidently drank 4 to 5 oz. of carbon tetrachloride from a champagne bottle. He died 12 days later. Autopsy disclosed acute hepatitis and nephritis. The lungs, which weighed 1,225 gm., showed hemorrhagic edema. The cut surface was mottled slate-red. Microscopic examination revealed moderate congestion, slight edema, purulent bronchopneumonia, and scattered pigmented macrophages.

Case 19 (A. F. I. P. No. 296993).—A white man aged 35 was repeatedly exposed to carbon tetrachloride in his work as a vacuum cleaner repair man. Hemoptysis, dyspnea, and cyanosis developed. An x-ray disclosed fluffy densities occupying the greater portion of each lung but sparing apices and bases. The heart was enlarged. There was increasing respiratory distress, with death 14 days after onset of the complaints and 6 hours after admission. Autopsy disclosed toxic hepatitis and nephritis. The lungs, which weighed 2,400 gm., showed marked edema and had a jelly-like consistency. Macroscopic examination revealed marked congestion and edema, focal hemorrhages, thickening of alveolar walls, early proliferation of alveolar lining cells, and numerous pigmented and nonpigmented macrophages.

Case 20 (A. F. I. P. No. 90402).—A white man aged 29 accidentally ingested a "mouthful" of carbon tetrachloride. Pulmonary edema was noted four days prior to death. He died 14 days after ingestion of the fluid and 9 days after admission. Autopsy disclosed acute hepatitis and nephritis. The lungs, which weighed 2,180 gm., showed hemorrhagic edema, were mottled reddish-purple, and were voluminous. Microscopic examination revealed marked congestion and edema, marked thickening of the alveolar walls, purulent bronchopneumonia, focal hemorrhages, hyaline membrane, proliferation of alveolar lining cells, fibroblastic proliferation, and aggregates of pigmented macrophages.

Case 21 (A. F. I. P. No. 157955).—A white man aged 39 drank 1 to 1½ oz. of carbon tetrachloride mistaken for whisky. He died 14 days after ingestion of the fluid and 12 days after admission. Autopsy disclosed acute hepatitis and nephritis. The lungs were voluminous, subcrepitant, and deep purple. They showed hemorrhagic edema and ill-defined areas of increased density and friable consistency. Microscopic examination revealed marked congestion and edema, thickening of alveolar walls with fibroblastic proliferation, hyaline membrane, and numerous pigmented macrophages.

CASE 22 (A. F. I. P. No. 239887).—A white man aged 31 cleaned a dance floor with carbon tetrachloride. X-ray examination showed obliteration of the right costophrenic angle and an enlarged heart. Pneumonic consolidation extended from both hilar regions, with a clear periphery. Marked respiratory distress with cyanosis developed despite oxygen therapy. He died 15 days after exposure and 12 days after admission. Autopsy disclosed toxic nephritis, hepatitis, myocarditis, and pneumonia. The lungs, which weighed 2,620 gm., were dark-red, had a meaty consistency, and showed edema. Microscopic examination revealed marked congestion and edema, focal hemorrhages, thickening of alveolar walls, proliferation of alveolar lining cells, hyaline membrane, fibrin within alveoli, purulent bronchopneumonia, fibroblastic proliferation, and organizing fibrin.

CASE 23 (A. F. I. P. No. 208127).—A white man aged 28 received 4 to 8 cc. of paraldehyde every four hours for six days. The paraldehyde was later found to contain 40% carbon tetrachloride. X-rays disclosed an enlarged heart with right-sided pulmonary edema. He died 15 days after the first ingestion of paraldehyde. Autopsy disclosed toxic nephritis and hepatitis. The lungs, which weighed 2,560 gm., were voluminous and heavy, with marked bloody edema and the consistency of firm gelatin. Microscopic examination revealed marked congestion and edema; alveoli filled with blood and fibrin, some of which was organizing; hyaline membrane; proliferation of alveolar lining cells; thickening of alveolar walls, and some fibroblastic reaction.

CASE 24 (A. F. I. P. No. 265462).—A white man aged 27, after a bout of heavy drinking, used an entire bottle of cleaning fluid to clean his clothes. He slept in a room without ventilation. Repeated episodes of pulmonary edema followed. X-rays showed "pneumonia" of the left hilar region. He died 15 days after exposure. Autopsy disclosed toxic nephritis and hepatitis. The lungs were voluminous and heavy, with hemorrhagic edema and a gelatinous consistency. Microscopic examination revealed moderate congestion and marked edema, fibrin within alveoli, purulent bronchopneumonia, hyaline membrane, thickening of alveolar walls, fibroblastic reaction, intrapulmonary hemorrhages, relatively few pigmented macrophages, and proliferation of alveolar lining cells.

CASE 25 (A. F. I. P. No. 485799).—A Negro aged 25, worked cleaning cables with carbon tetrachloride. Hemoptysis developed. X-rays disclosed an enlarged heart, with irregular density at the right base suggestive of pneumonia. He died 16 days after exposure. Autopsy disclosed acute nephritis and hepatitis. The condition of the lungs was not stated. Microscopic examination revealed marked congestion and edema, focal atelectasis, aspirated gastric contents, hyaline membrane, focal hemorrhages, proliferation of alveolar lining cells, and scattered macrophages, chiefly nonpigmented.

CASE 26 (A. F. I. P. No. 195415).—A white man aged 37, a fire fighter, was exposed to carbon tetrachloride fumes from a fire extinguisher over a period of months and immediately prior to admission. He died three days after admission. Marked pulmonary congestion, jaundice, and anuria had developed. Autopsy disclosed acute hepatitis and nephritis. The lungs, which weighed 2,200 gm., were large, heavy, beefy, and engorged. Microscopic examination revealed marked congestion and edema, focal atelectasis, purulent bronchopneumonia, organizing fibrin within alveoli, hyaline membrane, thickening of alveolar walls, some alveolar cell hyperplasia, and numerous pigmented histiocytes.

CASE 27 (St. Albans Naval Hospital No. LI-103).—A white man aged 26 worked in a closed space using a pressure spray of carbon tetrachloride to remove grease from machinery. He became acutely ill after an alcoholic bout. An admission x-ray of the chest was normal. Repeated bouts of pulmonary edema with bloody sputum occurred. He died 16 days after exposure and 13 days after admission. Autopsy disclosed acute nephritis and hepatitis and cardiac dilatation. The lungs, which weighed 2,960 gm., showed pleural hemorrhages. They were voluminous and heavy. The central portions were deeply congested, noncrepitant, meaty, and friable; the peripheral zones were emphysematous. Microscopic examination revealed marked congestion and edema, thickening of alveolar walls, focal hemorrhages, proliferation of alveolar lining cells, hyaline membrane, fibroblastic proliferation, and numerous pigmented macrophages.

Case Reports

BACTERIAL ENDOCARDITIS DUE TO PSEUDOMONAS AERUGINOSA

BURTON A. WAISBREN, M.D.
AND
EDWARD V. HASTINGS, M.D.
MILWAUKEE

THE PATHOGENICITY to man of the bacterial species Pseudomonas aeruginosa is well established.[1] This report and review of the literature concern the fifth proved case, with autopsy, of bacterial endocarditis due to Ps. aeruginosa.

REPORT OF A CASE

The patient was an 81-year-old white man who entered the Milwaukee County General Hospital with gross hematuria of six hours' duration. The history revealed that he had been hospitalized one month before with the same complaint. A retrograde pyelogram done at that time was normal, but Ps. aeruginosa was cultured from both ureters and the bladder.

The pertinent physical findings on admission were temperature 98.6 F., pulse rate 92 a minute, respirations 22 a minute, and blood pressure 114 mm. of mercury systolic and 66 mm. of mercury diastolic. The chest was clear. The heart was slightly enlarged to the left, and a Grade III systolic murmur was heard at the apex. This murmur had first been described six years previously during a hospital admission for unexplained hemoptysis. No tenderness or abnormal masses were present in the abdomen. Rectal examination was negative.

Laboratory studies revealed a hemoglobin content of 88% and a white blood cell count of 8,500 per cubic millimeter with 88% polymorphonuclear leucocytes. The nonprotein nitrogen was 58 mg. per 100 ml., and the blood Wassermann reaction was negative. The urine had a specific gravity of 1.005, was acid, and had a 4+ reaction for albumin. There were numerous red blood cells per high-power microscopic field. Roentgenograms of the chest revealed minimal cardiac enlargement and bilateral small pulmonary infiltrations.

Initial therapy consisted of the administration of sulfisoxazole (gantrisin®) 2 gm. a day, and penicillin, 400,000 units a day. Cystoscopy revealed dark bloody urine and mild cystitis. The day following admission the temperature rose to 102 F., and 1 gm. a day of streptomycin was added to the sulfisoxazole and penicillin. Administration of these three drugs was continued during the entire illness. The patient continued to have a daily temperature reading of 102 to 103 F. On the fourth hospital day his temperature was 103 F., his pulse rate 100 regular beats a minute, and his respirations 24 a minute. The blood pressure was 100/60, and the loud systolic murmur was still present. On the ninth hospital day the urine was still grossly bloody, the white blood cell count was 19,500 per cubic millimeter with 95% polymorphonuclear leucocytes, the serum bicarbonate was 23 mEq. per liter, the chlorides were 100 mEq. per liter, and the nonprotein nitrogen was 76 mg. per 100 ml. The output of urine ranged from 1,000 to 1,800 ml. a day.

From the Medical Service and Department of Pathology of the Milwaukee County General Hospital and the Departments of Medicine and Pathology of the Marquette University School of Medicine.

1. Robitzek, E. H., and Prausnitz, G.: Infection with the Bacillus Pyocyaneus (Pseudomonas Aeruginosa), Quart. Bull. Sea View Hosp. **8**:245, 1946. Stanley, M. M.: Bacillus Pyocyaneus Infections: Review Report of Cases and Discussions of Newer Therapy Including Streptomycin, Am. J. Med. **2**:253, 1947. Schaffer, A. J., and Oppenheimer, E. H.: Pseudomonas (Pyocyaneus) Infection of the Gastro-Intestinal Tract in Infants and Children, South. M. J. **41**:460, 1948. Yow, E.: Development of Proteus and Pseudomonas Infections During Antibiotic Therapy, J. A. M. A. **149**:1184, 1952.

The patient continued to have a daily temperature reading of 103 F. By the 17th hospital day his hemoglobin had fallen to 65%. His white blood cell count remained at 19,500 per cubic millimeter with 95% polymorphonuclear leucocytes. Blood drawn on the 17th hospital day grew Ps. aeruginosa on culture. On the 18th hospital day Ps. aeruginosa was again grown from the blood. At this time the patient did not appear gravely ill in spite of the continued spiking of his temperature. His output of urine remained between 1,400 and 1,800 ml. a day. On the 21st hospital day the patient appeared to be much worse. His blood pressure fell to 60/40, his heart sounds were very faint, and he presented the shock-like picture of bacteremia due to Gram-negative bacilli.[2] Nevertheless the output of urine remained above 1,000 ml. a day. One-half gram of oxytetracycline ("terramycin") was administered intravenously, without effect, 12 hours before death, which occurred late on the 21st hospital day. The strain of Ps. aeruginosa isolated from the blood stream was inhibited by 12 γ of aureomycin, 50 γ of chloramphenicol, 3 γ of oxytetracycline, 50 γ of streptomycin, 50 γ of penicillin, and 1.5 γ of polymyxin B per milliliter.

AUTOPSY FINDINGS

The heart weighed 400 gm. The mitral valve was the only valve to show any damage (Fig. 1). The auricular aspect of the posterior cusp presented an irregular nodular mass of

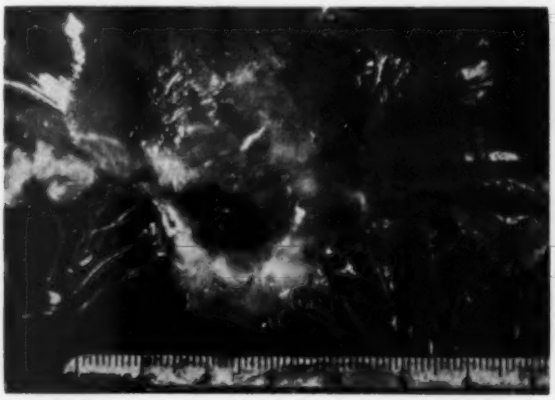


Fig. 1.—Gross appearance of ruptured mitral valve.

yellow and red, mottled, friable tissue which covered an area 12 by 18 mm. Separated from this lesion, and on the auricular endocardium, was friable, grayish, tan tissue 3 cm. in diameter. In addition there was an irregular perforation 3 mm. in diameter in the posterior cusp of the mitral valve (Fig. 1). This valve measured 9.5 cm. in circumference, and its margin and chorda tendineae showed nodular thickening.

The right kidney weighed 175 gm. and had a finely granular surface with several large depressed scars. There were zones of grayish-yellow discoloration in the region of the cortico-medullary junction. The left kidney weighed 215 gm. There was a yellow soft mass which measured 3 by 2.5 by 1.8 cm. on its anterolateral aspect. The bulk of this lesion consisted of necrotic material. In the upper portions of the medullary zone there were grayish-yellow softened areas which uniformly spared the tips of the papilla. The urinary bladder was thick walled, and its mucosa was congested and hemorrhagic.

Microscopic examination of the heart revealed brown and mucoid degeneration of the cardiac muscle. There was a destructive inflammatory process on the mitral valve, with red blood cells,

2. Waisbren, B. A.: Bacteria Due to Gram-Negative Bacilli Other Than the Salmonella, A. M. A. Arch. Int. Med. **88**:467, 1951.

Fig. 2.—High-power view of bacilli within substance of mitral valve.

fibrin, platelets, and polymorphonuclear leucocytes adhering to the eroded auricular surface. There were long basophilic bacilli both on the surface of the valve and within its substance (Fig. 2). Similar changes were seen in the lesion involving the auricular endocardium. A careful search revealed no phagocytosis by either monocytes or polymorphonuclear leucocytes. In the kidneys there was suppurative inflammation with abcess production at the corticomedullary junction. Sections of the coronary arteries showed atherosclerosis, but there was no invasion of the media of these vessels by bacilli. Sections of the liver were not remarkable. The splenic pulp was congested, and occasional areas of inflammatory reaction were visible.

The anatomic diagnoses were rheumatic heart disease, subacute bacterial endocarditis of the mitral valve and left atrium, rupture of the posterior cusp of the mitral valve, bilateral suppurative pyelonephritis, and papillary adenoma of the left kidney.

REVIEW OF LITERATURE

The ubiquity and invasiveness of Gram-negative bacilli make it necessary to set up rigid criteria for their acceptance as the etiologic agents in bacterial endocarditis. The three criteria used in this review were a clinical picture compatible with endocarditis, isolation of the organism from the blood stream at least several days before death, and demonstration of the bacilli within the heart valve by microscopic sections.

The first case of bacterial endocarditis due to Ps. aeruginosa that met these criteria was reported by Rolly in 1906.[3] The patient was a 28-year-old woman whose 28-day illness resembled typhoid. Antemortem blood and spinal fluid cultures showed Ps. aeruginosa, and autopsy revealed endocarditis of the mitral valve, meningitis, and multiple metastic abcesses. There were bacilli both within the heart valves and within walls of the coronary arteries.

Kearns reported the next case in 1936.[4] The disease occurred in a 33-year-old heroin addict and ran a six-week course. During this time the leucocyte count remained low and the hemoglobin content fell to 55%. Autopsy showed involvement of both the aortic and the mitral valve. There was invasion of the aortic and pulmonary arteries by the bacilli. In addition there was marked phagocytosis of Ps. aeruginosa by monocytes.

The striking feature of the next case, which was reported by Fish, Hand, and Keim,[5] was invasion of the walls of the arteries by Ps. aeruginosa. These authors noted that there was no phagocytosis of the invading bacilli by polymorphonuclear leucocytes but did not mention the phagocytosis by monocytes which was described by Kearns.[4] The patient was a 71-year-old man who had been repeatedly catheterized. His leucocyte count remained at 9,000 per cubic millimeter, and his hemoglobin dropped from 75% to 55% during the course of the illness.

Moragues and Anderson's[6] report of the fourth case of endocarditis appeared in 1943. The patient, a 66-year-old man, was a diabetic with rheumatic heart disease who had been catheterized repeatedly. Vegetations on the mitral valve and focal

3. Rolly: Pyozyaneussepsis bei Erwachsenen, München. med. Wchnschr. **53**:1399, 1906.

4. Kearns, J. J.: Malignant Endocarditis Due to Bacillus Pyocyaneus, Arch. Path. **21**:839, 1936.

5. Fish, G. W.; Hand, M. M., and Keim, W. F., Jr.: Acute Bacterial Endocarditis Due to Pseudomonas Æruginosa, Am. J. Path. **13**:121, 1937.

6. Moragues, V., and Anderson, W. A. D.: Endocarditis Due to Pseudomonas Aeruginosa, Ann. Int. Med. **19**:146, 1943.

glomerulonephritis were found at postmortem examination. In addition there was invasion of the walls of the arteries by the bacilli that was similar to that described in the other three proved cases.

Four additional cases were found in which endocarditis may have been caused by Ps. aeruginosa but in which our arbitrary criteria were not fulfilled.[7] The patients ranged in age from 2½ to 65 years, and in each instance the mitral valve was involved.

Successful treatment of a case of bacterial endocarditis due to Ps. aeruginosa recently was reported by Kenoyer, Stone, and Levin.[8] The patient was a 20-year-old man with rheumatic heart disease whose blood was sterilized by parenteral administration of neomycin.

COMMENT

The clinical course, the repeated antemortem isolation of the organism, and the autopsy findings conclusively establish this case as one of bacterial endocarditis due to Ps. aeruginosa. It differed from the other reported cases in that no invasion of the blood vessels by the bacilli was found.[9] Such invasion may have been prevented by the chemotherapy. There was, however, the notable lack of phagocytosis of the organism by polymorphonuclear leucocytes that was mentioned in several of the other reports.[10] This lack probably was due to the fact that resistance to Gram-negative organisms is usually mediated through humoral rather than cellular mechanisms.

SUMMARY

A case of bacterial endocarditis due to Ps. aeruginosa and a review of four other cases of endocarditis due to this organism have been presented. The outstanding features of this case were a clinical course characteristic of bacteremia due to Gram-negative bacilli, rupture of the posterior cusp of the mitral valve, and lack of phagocytosis by polymorphonuclear leucocytes.

7. (*a*) Blum, S.: Ein Fall von Pyocyaneus Septichämie mit komplizcerender Pyocyaneus Endocarditis im Kindesalter, Centralbl. Bakt. **25**:113, 1899. (*b*) de la Camp: Zur Kenntnis der Pyocyaneusepsis, Charité-Ann. **28**:92, 1903. (*c*) Thayer, W. S.: Studies on Bacterial (Infective) Endocarditis, Johns Hopkins Hosp. Rep. **22**:1, 1926. (*d*) Büngeler, W.: Ueber Endocarditis maligna durch Bazillus pyocyaneus, Frankfurt. Ztschr. Path. **35**:428, 1927.

8. Kenoyer, W. L.; Stone, C. T., and Levin, W. C.: Bacterial Endocarditis Due to Pseudomonas Aeruginosa—Treated with Neomycin, Am. J. Med. **13**:108, 1952.

9. Kearns.[4] Fish, Hand, and Keim.[5] Moragues and Anderson.[6] Blum.[7a]

10. Fish, Hand, and Keim.[5] Moragues and Anderson.[6]

PYLORIC OBSTRUCTION DUE TO MUCOSAL DIAPHRAGM

ALEXANDER N. ROTA, M.D.
OTTAWA, ONT., CANADA

PYLORIC obstruction due to a mucosal diaphragm, although described in the standard reference textbooks,[1] is exceedingly rare. Because of the rarity of this condition, it is thought worth while to record the present case, which is believed to be the third reported in the medical literature.

REPORT OF A CASE

The patient, a woman of 74 years, complained of intermittent attacks of nausea and vomiting followed by diarrhea over a period of eight years. These attacks, which always followed the same pattern and which were not related to diet or emotional states, occurred in the late evening or early morning. The vomitus consisted of undigested food and did not contain bile. She had sought medical advice on several occasions, and roentgenographic investigations had been done in 1942 and 1944. The first report was "pyloric ulcer with partial obstruction," and the second was "pyloric obstruction with 25% retention in 24 hours, thickening of the pylorus due to either scirrhous carcinoma or scar tissue of healed ulcer."

No surgical procedures were undertaken, and the patient carried on as before, under various forms of medical treatment, without obtaining much relief.

She was admitted to the Ottawa General Hospital in 1951, by which time she had come to regard herself as a "neurotic." Physical examination was unremarkable, although the patient stated she had lost 21 lb. (9.53 kg.) in weight during the previous eight months. The results of laboratory investigation, which included urinalysis, hemogram, and gastric analysis, were within normal limits. Examination of the gastric contents for cancer cells was negative. Repeated barium series revealed only an "atonic and ptosed stomach with no evidence of ulcer or new growth in the stomach or duodenum." Barium enema and sigmoidoscopic examination revealed no abnormalities. The patient showed no evidence of mental instability.

Partial gastrectomy was done, after which the patient made an uneventful recovery, and 13 months later the patient was reported to have gained weight and to be free of symptoms.

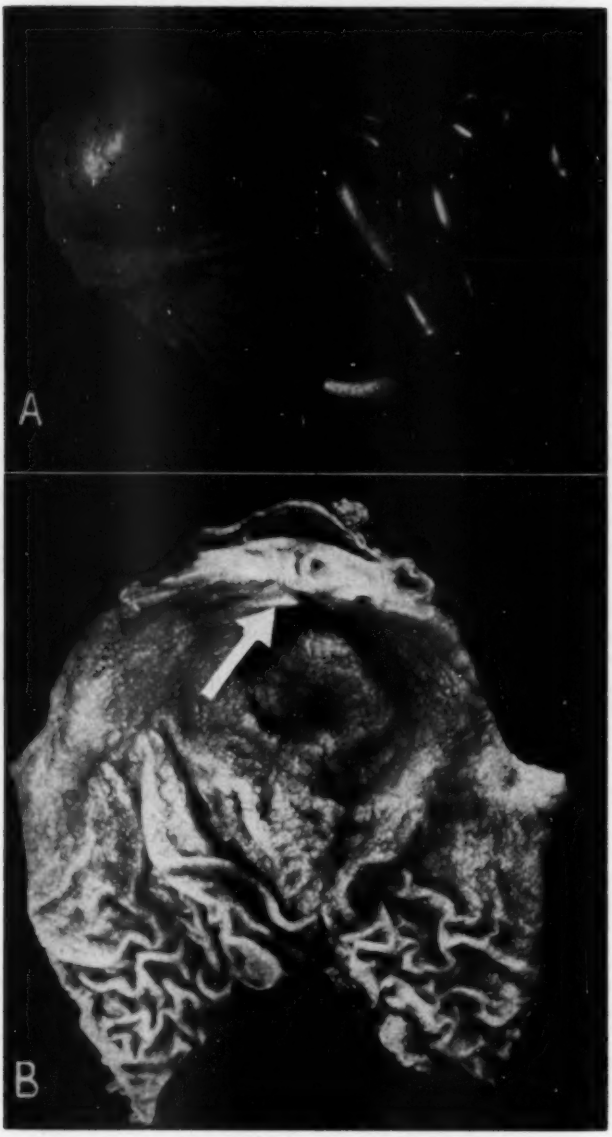
PATHOLOGICAL REPORT

The specimen consisted of a portion of stomach including the pylorus and an attached cuff of duodenum. The stomach measured 15 cm. along the greater curvature and 8 cm. along the lesser curvature. The attached portion of duodenum was 1.5 cm. in length. The pyloric musculature was thickened into a firm but wide ring. A finger inserted into the pylorus did not pass through but demonstrated the presence of a mucosal diaphragm. When the diaphragm was pushed into the

Dr. Rota is now in the Department of Pathology, McGill University, Montreal, Canada.

From the Department of Pathology, University of Ottawa, and the Ottawa General Hospital.

1. Anderson, W. A. D., Editor: Pathology, St. Louis, C. V. Mosby Company, 1948, p. 824. Arey, L. B.: Developmental Anatomy, Ed. 5, Philadelphia, W. B. Saunders Company, 1941, pp. 215-216.



A, a view of the stomach showing the pyloric mucosal diaphragm with its small central aperture. A finger has been placed in the duodenum, the mucosal diaphragm has been pushed into the stomach, and the stomach has been everted. *B*, stomach opened to show the pebbled appearance of the mucosa in the pyloric antrum. The arrow points to the mucosal diaphragm, opened through its central aperture.

stomach and the stomach then everted over the finger, a small opening approximately 0.4 cm. in diameter was discovered in the center of the diaphragm (Fig., *A*). When the specimen was opended along the greater curvature, the mucosa of the pyloric portion was seen to be reddened, flattened, and finely pebbled in appearance (Fig., *B*). This was in sharp contrast to the paler rugose fundic mucosa. The diaphragm appeared to be formed of a circumferential fold of redundant mucosa. The duodenal mucosa was unremarkable.

Microscopic sections were taken to include the diaphragm and underlying pyloric sphincter. The mucosa showed an increase in fibrous tissue in the lamina propria and a moderate lymphocytic infiltration. The muscularis mucosae was thickened by hypertrophy of muscle cells and by fibrosis. A lymphocytic infiltration also involved this layer. The submucosa was increased in thickness. It was loose, fatty, and infiltrated with a few lymphocytes. The pyloric musculature showed slight hypertrophy. There was a complete absence of scarring. Numerous sections from the pyloric portion of the stomach showed a well-marked chronic gastritis. Here there was a marked infiltration of the lamina propria by lymphocytes. The glands were moderately reduced in size, depth, and number, and the muscularis mucosae was thickened by fibrosis. The deeper coats were unremarkable. Sections of the fundic portion showed no evidence of gastritis.

Pathological Diagnosis.—The pathological diagnoses were (1) obstruction of pylorus by a mucosal diaphragm; (2) chronic antral gastritis.

COMMENT

Consideration was given to the possibility that the pyloric mucosal diaphragm represented an unusual form of prolapse of the gastric mucosa into the duodenum. There are many reports of this condition,[2] and in some of them there is mention of a form of prolapse involving the entire circumference of the pyloric and prepyloric mucosa. However, a permanent diaphragm-like structure having a small central aperture has not been described, and it is difficult to see how such a deformity could have developed on the basis of a circumferential mucosal prolapse.

Despite the earlier roentgenological findings, no evidence of past or present ulceration was present in the pathological specimen, and it did not seem possible to relate the lesion to chronic gastric ulceration. The chronic antral gastritis and slight hypertrophy of pyloric musculature were regarded as being secondary to the pyloric obstruction.

A search of the medical literature reveals reports of two cases in which there were pyloric obstructions due to mucosal diaphragms. In 1940 Touroff and Sussman [3] reported a unique case of congenital gastric obstruction in a newborn infant

2. Eliason, E. L., and Wright, V. W. M.: Benign Tumors of the Stomach, Surg., Gynec. & Obst. **41**:461-472, 1925. Golden, R.: Antral Gastritis and Spasm, J. A. M. A. **109**:1497-1500, 1937. Kaplan, I. W., and Shepard, R. M.: Prolapse of the Gastric Mucosa into the Duodenum, ibid. **147**:554-560, 1951. MacKenzie, W. C.; Macleod, J. W., and Bouchard, J. L.: Transpyloric Prolapse of Redundant Gastric Mucosal Folds, Canad. M. A. J. **54**:553-558, 1946. Manning, I. H., Jr., and Gunter, J. U.: Prolapse of Redundant Gastric Mucosa Through the Pyloric Canal into the Duodenum, Am. J. Path. **26**:57-73, 1950. Rees, C. E.: Prolapse of the Gastric Mucosa Through the Pylorus, Surg., Gynec. & Obst. **64**:689-694, 1937.

3. Touroff, A. S. W., and Sussman, R. M.: Congenital Prepyloric Membranous Obstruction in a Premature Infant, Surgery **8**:739-755, 1940.

treated successfully by surgical means. The obstruction was caused by a complete prepyloric septum of mucous membrane. They discussed two theories, either of which they believed might explain the presence of this complete septum. The first theory was that the septum was formed as a result of fusion of folds of mucous membrane, which in this region normally are reduplicated to form the pyloric valve. The second theory was that the septum represented the result of an abnormally high closure of the duodenum in the embryological state with subsequent incomplete fusion of vacuoles to reestablish the lumen. Sames,[4] in 1949, described the finding of a prepyloric mucosal diaphragm in a stomach removed in treatment of a chronic gastric ulcer in a 40-year-old woman. The diaphragm, which was situated in the antrum 1.5 cm. from the pylorus, measured 3 mm. in thickness and had a small eccentric opening, measuring 3 mm., which could be dilated to 5 mm. The chronic gastric ulcer on the lesser curvature was apparently unrelated. The possibility of mucosal prolapse being an etiological factor was considered here, but Sames favored the view that the mucosal diaphragm was congenital in origin.

In the present case also it does not seem possible to exclude a congenital origin for the lesion, despite the late onset of symptoms and the advanced age of the patient. To explain this late onset and the intermittent character of the symptoms, one might postulate that the small aperture was to some extent expansible during life. Possibly, also, there was no necessity for a pyloric passage larger than that provided by the orifice of the diaphragm, except when the stomach contents contained large lumps of insufficiently masticated food. In this regard, it is interesting that in Sames's case obstructive symptoms were not pronounced, despite the small size of the diaphragmatic aperture.

SUMMARY

A case is reported of intermittent obstruction due to a mucosal diaphragm with a small central opening, situated at the pylorus, in a woman of 74 years. Treatment was by gastric resection. This is apparently the third case of pyloric obstruction by a mucosal diaphragm to be reported in the medical literature. In the two previously recorded cases, one that of a 6-day-old infant, the other, that of a woman of 40 years, the obstruction was prepyloric in position. The pathogenesis of the lesion in this case cannot be established with certainty.

4. Sames, C. P.: A Case of Partial Atresia of the Pyloric Antrum Due to a Mucosal Diaphragm of Doubtful Origin, Brit. J. Surg. **37**:244-246, 1949.

General Reviews

HUMAN SIGNIFICANCE OF EXPERIMENTAL CARCINOGENESIS

PAUL E. STEINER, M.D.
CHICAGO

THE SUBJECT "Human Significance of Experimental Carcinogenesis" was selected for this article because much information recently coming from experimental laboratories appears to be applicable to the human problem. Until very recently, when statisticians, industrial hygienists, and others entered the field, all original observations on carcinogens in man were made by practicing physicians who were familiar with their patient and his environment. Probably many additional carcinogen-to-cancer relationships remain to be discovered by physicians aware of the problems.

In the last two decades approximately 400 chemical compounds having cancer-inducing activity in experimental animals have been reported. Of these, 357 were summarized by Hartwell [1] through 1947, and the remainder have been described since then. Some physical and biological carcinogens are also known. In experimental carcinogenesis the results are constant, reproducible, and predictable. Recent experiments have revealed much new information on the nature of carcinogens and on the mechanism of carcinogenesis. I am here concerned with the significance of this work in relation to the human problem. The chemical aspects of the carcinogens themselves will not be covered, because excellent reviews on this subject are available.[2] For present purposes it should be recognized that agents inducing tumors in man are also known, that most of them are chemicals, that almost all of them have had experimental confirmation in animals, and that a far greater number of carcinogens are known for experimental animals than for man, leading to the suspicion that many more remain to be discovered for the human.

The numerous cancer-producing agents have been excellent experimental tools for the study of carcinogenesis, so that today much is known about the conditions of transformation of normal to cancer cells. Although the essential nature of this change and of the resulting neoplastic cell still eludes full understanding, the condi-

From the Department of Pathology, the University of Chicago.

Read before the Section on Pathology and Physiology at the One Hundred First Annual Session of the American Medical Association, Chicago, June 11, 1952.

1. Hartwell, J. L.: Survey of Compounds Which Have Been Tested for Carcinogenic Activity, Ed. 2, Federal Security Agency, U. S. Public Health Service, 1951.

2. Cook, J. W., and Kennaway, E. L.: Chemical Compounds as Carcinogenic Agents: Second Supplementary Report; Literature of 1938 and 1939, Am. J. Cancer **39**:381, and 520, 1940. Fieser, L. F.: Carcinogenic Activity, Structure, and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons, ibid. **34**:37, 1938. Badger, G. M.: The Carcinogenic Hydrocarbons: Chemical Constitution and Carcinogenic Activity, Brit. J. Cancer **2**:309, 1948. Haddow, A., and Kon, G. A. R.: Chemistry of Carcinogenic Compounds, Brit. M. Bull. **4**:314, 1947. Hieger, I.: Chemical Carcinogenesis: A Review, Brit. J. Indust. Med. **1**:1, 1949. Hartwell.[1]

tions of its occurrence are well known—so well known that it is now possible to formulate principles of carcinogenesis, at least provisionally. New concepts have been introduced, characterized by new terms which are rapidly becoming incorporated into medical terminology and thinking about cancer. This paper deals primarily with these principles as they correlate human and experimental experience. It is not intended to be an exhaustive review of experimental carcinogenesis. The interested reader is referred to reviews of this subject by Berenblum,[3] Morton,[4] Rous,[5] Rusch,[6] and Spencer.[7]

The knowledge obtained from experimental carcinogenesis can be divided into three broad categories in relation to the human problem: Some of the observations appear to have a direct human counterpart and can be used to interpret this counterpart. A larger number of observations probably have an equivalent in man, although such an equivalent is not yet recognized; promising research problems are suggested by this category. A third class probably has no human analogy because the experimental work has been done under conditions exaggerated or distorted beyond what is seen in the human. Examples are the work with highly inbred strains of mice, with massive doses of highly potent carcinogenic chemicals, and with highly artificial alterations of the hormone balance. Experiments involving such conditions can, nevertheless, have significance for human cancer problems because they may by their exaggeration reveal cause-and-effect relationships which would otherwise, being marginal, escape attention.

In man a number of chemical, physical, and biological carcinogens have been recognized.[8] Nevertheless, these agents together account for only a small proportion of the estimated nearly 300,000 new cases of cancer that occur each year in this country.[9] The remainder are said to be "spontaneous," by which is meant that the causes are not yet recognized. The principles learned from studies on carcinogens and in experimental carcinogenesis, to be discussed hereafter, indicate very strongly that at least some if not most of the so-called spontaneous human cancers may be due to unrecognized carcinogens. They also indicate where and how to search for human carcinogens. Advance in knowledge on the etiology of human cancer should be greatly furthered by widespread appreciation of the underlying principles by alert clinicians who have close contact with their patients.

The tumor deliberately induced in experimental carcinogenesis from the skin, subcutaneous tissues, or other sites originates after an interval known as the "induction time" or "latent period" from a cell or group of cells exposed to the inducer

3. Berenblum, I.: Irritation and Carcinogenesis, Arch. Path. **38**:233, 1944.

4. Morton, J. J.: The Cancer Problem, J. A. M. A. **135**:957, 1947.

5. Rous, P.: The Nearer Causes of Cancer, Barnard Hospital Lecture, J. A. M. A. **122**:573, 1943; Concerning the Cancer Problem, Am. Scientist **34**:329, 1946.

6. Rusch, H. P.: Extrinsic Factors That Influence Carcinogenesis, Physiol. Rev. **24**:177, 1944.

7. Spencer, R. R.: The Meaning of Cancer Research, J. A. M. A. **137**:1361, 1948.

8. (*a*) Hueper, W. C.: Occupational Tumors and Allied Diseases, Springfield, Ill., Charles C Thomas, Publisher, 1942; (*b*) Environmental and Occupational Cancer, Pub. Health Rep., Supp. 209, p. 1, 1949. (*c*) Henshaw, P. S.: Implications from Studies with Physical Carcinogens, J. Nat. Cancer Inst. **5**:419, 1945. (*d*) Cramer, W.: The Origin of Cancer in Man, J. A. M. A. **119**:309, 1942.

9. Steiner, P. E.: An Evaluation of the Cancer Problem, Cancer Res. **12**:455, 1952.

either at the point where the agent is applied or at that to which it is transported in the body. Thus the tumor may arise at points of application, metabolism, storage, or excretion, the site depending mainly on the carcinogen itself. It arises from the direct local action on the cell, as is indicated by the induction of cancer in tissue culture.[10] However, the ease or difficulty with which the normal cell is transformed to a neoplastic cell to initiate a tumor is determined in part by general influences, including hereditary, hormonal, and nutritional factors. Indeed it appears that these modifying factors may themselves determine, partially or completely, the adequacy of carcinogens; if they are very potent, they perhaps are sufficient themselves to start a tumor, as may be the case with congenital tumors.

The induction of tumors is not a simple process. It has been subdivided into several named stages, each of which has distinct attributes.[11] Corresponding steps have not yet all been recognized in man. Once started, a tumor is independent of the continued action of the inducing agent, according to the tissue-culture work of Lewis. It is said to be autonomous. This is not to say that the growth cannot be influenced, as indeed it can. It is important to distinguish between the induction of a tumor and its subsequent growth. I am here concerned with the former.

The intracellular mechanism distinctive of cancer is self-perpetuating. The numerous theories as to what endows the neoplastic cell, benign or malignant, with the new properties which characterize its state include mutation, virus, metabolism, and other concepts. The causes of some human cancers are known in the sense that influences or agents have been recognized which can act on normal cells to convert them to neoplastic cells. The exact nature of the cellular aberration, the reasons for its vigorous and uncontrolled behavior, and the explanation for its continuation are not yet known.

Some outstanding principles of experimental carcinogenesis are discussed in the following subsections in the light of their possible significance in the human problem. The potential utility of these concepts to the informed practicing physician now appears enormous. In the past, most of the known human carcinogen-to-tumor relationships were discovered by clinicians, and it is probable that this type of progress will continue.

PRINCIPLES OF EXPERIMENTAL CARCINOGENESIS IN RELATION TO CANCER IN MAN

Morphology of Carcinogenesis.—The change in the cell from normal to neoplastic is preceded by a series of important events which are only partly understood

10. Earle, W. R.: Changes Induced in a Strain of Fibroblasts from a Strain C3H Mouse by the Action of 20-Methylcholanthrene, J. Nat. Cancer Inst. **3**:555, 1943. Firor, W. M., and Gey, G. O.: Observations on Conversion of Normal into Malignant Cells, Ann. Surg. **121**:700, 1945.

11. (*a*) Rusch, H. P., and Kline, B. E.: Further Evidence for Successive Stages in the Formation of Neoplasms, Arch. Path. **42**:445, 1946. (*b*) Berenblum, I., and Shubik, P.: A New Quantitative Approach to the Study of the Stages of Chemical Carcinogenesis in the Mouse's Skin, Brit. J. Cancer **1**:383, 1947. (*c*) Mottram, J. C.: A Developing Factor in Experimental Blastogenesis, J. Path. & Bact. **56**:181, 1944. (*d*) Tannenbaum, A.: The Importance of the Differential Consideration of the Stages of Carcinogenesis in the Evaluation of Cocarcinogenic and Anticarcinogenic Effects, Cancer Res. **4**:678, 1944.

in the biological [12] and chemical sense.[13] The eventual actual change is recognized morphologically as being fairly abrupt; a long stage of preceding inflammation formerly observed [14] is now known to be unessential.[15] It is not seen when cancer is formed in tissue culture.[16] If minimal and not excessive cell-necrotizing doses of the agent are used, evidence of cell damage and inflammation are inconspicuous or absent in most tissues and with most agents.[17]

The morphological events differ somewhat on epithelial surfaces,[18] in glandular and other internal organs,[19] and in the connective tissues.[20] In general there is a slight stimulation of the cells from which the tumor ultimately arises, followed eventually by changes in cell morphology, chiefly nuclear, after which the altered cells proliferate more rapidly as tumor cells. In some instances an important stage of atrophy immediately precedes the transformation to neoplasm. This observation is true particularly in the neoplasms induced by hormones, according to Gardner,[21] and in leukemia.[22] In other sites, particularly in glandular organs such as the prostate and salivary gland, the carcinogen induces first metaplasia and then neoplasia from the metaplastic cells.[19] In any event the tumor which is induced originates from the affected cells and is of a morphological type corresponding to those cells. Thus sarcoma of many types, carcinoma of many kinds, leukemia, and other tumors may be induced by the same agent, according to the type of cell exposed. Only a few carcinogenic agents are specific for one type of cell. It is impossible from the microscopic appearance of the tumor to name the carcinogen.

The morphogenesis of human neoplasms, so far as it is known, resembles that in the experimental animal. In both situations, actinic tumors of the skin undergo

12. Rusch.[6] Berenblum and Shubik.[11b] Tannenbaum.[11d]

13. (*a*) Miller, E. C.: Studies on the Formation of Protein-Bound Derivatives of 3,4-Benzpyrene in the Epidermal Fraction of Mouse Skin, Cancer Res. **11**:100, 1951. (*b*) Cowdry, E. V.: Microscopic and Chemical Properties of Precancerous Lesions, Science **102**:165, 1945.

14. (*a*) Beck, S.: On the Failure of Acute and Subacute Inflammation to Influence Carcinogenesis with 3:4-Benzpyrene, Brit. J. Exper. Path. **19**:319, 1938. (*b*) Orr, J. W.: An Investigation of the Histological Changes in the Subcutaneous Tissues of Mice During the Induction of Sarcoma by Carcinogenic Hydrocarbons, J. Path. & Bact. **49**:157, 1939. (*c*) Wolbach, S. B.: Responses to Carcinogenic Chemical Antecedent to Tumor Formation, Am. J. Path. **13**:662, 1937.

15. Peacock, P. R., and Beck, S.: Rate of Absorption of Carcinogens and Local Tissue Reaction as Factors Influencing Carcinogenesis, Brit. J. Exper. Path. **19**:315, 1938. Beck.[14a]

16. Nettleship, A.: Morphology of Sarcomas Derived from Fibroblasts Previously Treated with 20-Methylcholanthrene in Vitro, J. Nat. Cancer Inst. **3**:559, 1943.

17. Grady, H. G., and Stewart, H. L.: Origin of Induced Pulmonary Tumors in Strain A Mice, Pub. Health Rep. **55**:169, 1940.

18. Grady, H. G.; Blum, H. F., and Kirby-Smith, J. S.: Types of Tumor Induced by Ultraviolet Radiation and Factors Influencing Their Relative Incidence, J. Nat. Cancer Inst. **3**:371, 1943. Glücksmann, A.: The Histogenesis of Benzpyrene-Induced Epidermal Tumors in the Mouse, Cancer Res. **5**:385, 1945. Cowdry.[13b]

19. Steiner, P. E.: Comparative Pathology of Induced Tumors of the Salivary Glands, Arch. Path. **34**:613, 1942.

20. Orr, J. W.[14b] Oberling, C.; Guerin, M., and Guerin, P.: Particularités évolutives des tumeurs produites avec de fortes doses de benzopyrène, Bull. Assoc. franc. étude cancer **28**:198, 1939.

21. Gardner, W. U.: Hormonal Imbalances in Tumorigenesis, Cancer Res. **8**:397, 1948.

22. Kaplan, H. S., and Brown, M. S.: Effect of Peripheral Shielding on Lymphoid Tissue Response to Irradiation in C57 Black Mice, Science **116**:195, 1952.

the same evolution, malignant lymphomas follow a stage of atrophy,[23] osteogenic sarcoma may develop in radiation osteitis,[24] liver tumors follow cirrhosis,[25] bladder cancer usually develops from a papillary lesion, and so on.

The morphology of human cancers and of tumors produced in animals is often similar, although species differences are sometimes seen. The tumors may be histologically indistinguishable. It is possible to substitute experimental cancers for their "spontaneous" human counterpart for projection at conferences without danger of detection by the audience. There are many reasons—morphological, chemical, biological, etiological—to believe that they are essentially the same disease process.

The hyperplastic changes in epidermis of experimental animals produced by carcinogenic agents resemble superficially those produced by noncarcinogenic agents. However, more precise cytological studies reveal a difference.[26] Similar studies have not been made on man's epidermis, but they ought to be, since they might distinguish between preneoplastic and harmless hyperplasia and thus aid in precise diagnosis of precancerous epithelial lesions—something which is now sometimes not possible except on a crude statistical basis of probability. In the stratified squamous epithelium of the uterine cervix, changes induced by infection and hormones may simulate those of early neoplasm, leading at times to confusion in diagnosis.

Once the biological change from normal to neoplastic has occurred in a cell or group of cells, the subsequent outgrowth of these cells from a microscopical lesion into a mass recognized as tumor is determined by a new set of factors, with which I am here only secondarily concerned. The initiation, induction, or origination of a tumor must be distinguished from its subsequent growth. The cells which compose the tumor are largely, if not entirely, the descendants of those that were formed at its induction. The tumor probably does not grow by progressive cancerization of the surrounding normal cells, except, perhaps, in the case of some of the experimental virus-induced tumors. The microscopical appearance of apparent progressive cancerization of normal cells and their incorporation into a primary tumor, sometimes seen in epithelial surfaces,[27] is probably an erroneous interpretation of events, since it is found also when tumors metastasize into such surfaces. This question of growth by progressive cancerization of normal cells should be sharply separated from that of multicentric origin of tumors, synchronous or serial, from previously treated surfaces; unfortunately these two concepts are sometimes confused, to the detriment of both.

---

23. Block, M., and Jacobson, L.: Development of Acute Leukemia in Human Adults, Cancer Res. **12**:250, 1952.

24. Martland, H. S.: The Occurrence of Malignancy in Radio-Active Persons: General Review of Data Gathered in Study of Radium Dial Painters, with Special Reference to Occurrence of Osteogenic Sarcoma and Interrelationship of Certain Blood Diseases, Am. J. Cancer **15**:2435, 1931.

25. Berman, C.: Primary Carcinoma of the Liver: A Study in Incidence, Clinical Manifestations, Pathology, and Ætiology, London, H. K. Lewis & Co., Ltd., 1950.

26. Salaman, M. H., and Gwynn, R. H.: The Histology of Co-Carcinogenesis in Mouse Skin, Brit. J. Cancer **5**:252, 1951.

27. Willis, R. A.: The Mode of Origin of Tumors: Solitary Localized Squamous Cell Growths of Skin, Cancer Res. **4**:630, 1944.

Nature of Carcinogens.—The experimental carcinogenic agents are classified as physical, biological, and chemical. The chemical, in turn, may be inorganic or organic and aromatic or aliphatic. The molecular structure ranges from simple (i. e., beryllium, zinc, urethan) to complex (i. e., 9,10-dimethyl-1,2-benzanthricene, 3,4-benzpyrene, diethylstilbestrol). Carcinogens can be classified in nature as normal or as abnormal constituents of the body and in origin as endogenous [28] or exogenous.

In man also the known chemical carcinogens are simple (i. e., arsenic, chromates, benzol) or complex (i. e., 3,4-benzpyrene in soot and tar). Of greater importance is the observation that they include some substances that are unusual and possibly others that are common in man's environment. In the past the former have been easily recognized as carcinogens, but agents which are common and widespread in the environment and of a low potency have been extremely difficult to prove. The current difficulties in establishing the etiological factors in lung cancer demonstrate this observation. Certain mine dusts in the Sudeten mountains, nickel, chromates, asbestos, tar, and possibly arsenic have been incriminated as inducers of human lung cancer, together with other crude mixtures, such as soot, tar, and oils in which the active ingredient is not known.[29] Left unexplained are the causes in the great majority of cases. Some of these may well be other unusual chemicals and rare exposures as yet unrecognized, but the majority are probably common agents, perhaps in combinations, to which most persons are exposed, such as 3,4-benzpyrene in soot [30] and in other atmospheric dusts [31] and other agents.[32]

It appears to be a historical fact that when exposure of a human being was to a carcinogen of an uncommon type and when the resulting tumor was in an unusual site, the cause-and-effect relationship was readily apparent. But if exposure was to a common type of agent (perhaps at low intensity or potency, so that the induction time was long) and the resulting neoplasm one of the common varieties (i. e., of the stomach, colon, or lung), then the relationship might entirely escape attention or become apparent only by special methods of study. Such methods include statistical and experimental techniques.

The situation with regard to the etiology in man is further complicated by the nature of the chemicals. They can be divided into two groups, of which one consists of carcinogens of known chemical structure and the other of complex mixtures of compounds in which the active ingredient is not known. While 3,4-benzpyrene and other hydrocarbons have been identified in soot,[30] the active components in

28. Hieger, I.: Carcinogenic Activity of Lipoid Substances, Brit. J. Cancer **3**:123, 1949. Steiner, P. E.; Stanger, D. W., and Bolyard, M. N.: Comparison of the Carcinogenic Activity in Extracts of Human Liver and Other Human and Animal Organs, Cancer Res. **7**:273, 1947.

29. Hueper, W. C.: (*a*) Environmental Lung Cancer, Indust. Med. **20**:49, 1951; (*b*) footnote 8*a*.

30. (*a*) Waller, R. E.: The Benzpyrene Content of Town Air, Brit. J. Cancer **6**:8, 1952. (*b*) Falk, H. L., and Steiner, P. E.: The Identification of Aromatic Polycyclic Hydrocarbons in Carbon Blacks, Cancer Res. **12**:30, 1952.

31. Falk, H. L.; Steiner, P. E.; Goldfein, S.; Breslow, A., and Hykes, R.: Carcinogenic Hydrocarbons and Related Compounds in Processed Rubber, Cancer Res. **11**:318, 1951.

32. Haerem, A. T.: Carcinogenic Effect of Sulfonamides, Proc. Soc. Exper. Biol. & Med. **68**:330, 1948.

petroleum products,[33] mine dusts,[34] tobacco tars,[29a] and other agents have not been isolated.

It is of more than passing interest that many carcinogenic chemicals are under some conditions inhibitors of tumor growth.[35] Conversely, many inhibitors of tumor growth are carcinogenic. This phenomenon is illustrated by the therapeutic agents x-rays, radium, and radioactive iodine, all of which have been reported as carcinogenic under special conditions of testing. Also of interest is the observation that while some estrogens are carcinogenic in animals, some of the carcinogens are estrogenic. Estrogens may be regarded as specific growth stimulants. Perhaps of closer relation to the cancer problem is the recorded fact that many carcinogens are mutagenic, while other closely related carcinogenic chemicals are not.[36] It is recalled that one theory of the nature of cancer is that it is a mutation. The action of carcinogens in causing metaplasia has already been mentioned. It is clear that carcinogenicity is closely related to other important biological phenomena, including growth, estrogenicity, mutagenicity, and ability to cause metaplasia.

In experimental animals, some virus-like agents induce tumors. Outstanding examples are Bittner's mammary-tumor agent in mice, the Shope rabbit-papilloma virus, Lucké's frog renal-carcinoma virus, and the Rous chicken-sarcoma agent. There is no known human counterpart to the animal virus tumors at the present time.

Latent Period or Induction Time.—The period that elapses between the first exposure to a carcinogenic agent and the appearance of the resulting tumor is designated the latent period or the induction time. It varies in length with agent, dosage, tissue or organ, species, and other factors. Conversion of cell from normal to neoplastic requires a definite period, which cannot be reduced below a certain minimum regardless of the amount of agent employed. An exception to this statement is provided by the example of some virus-induced tumors in fowls which appear quickly, showing that the method of induction is different from that with chemical or physical carcinogens. The latent period represents a certain proportion of the biological life span of each species. It averages about 10 to 15% of that span, although it may be longer or shorter. Common induction times are 5 months in mice, 8 months in rats, 12 months in guinea pigs, 24 months in dogs, and 5 to 15 years in man.

Important events are taking place in the cells during the latent period.[37] This period is not explained simply as the interval required for newly formed cancer

33. Smith, W. E.; Sunderland, D. A., and Sugiura, K.: Experimental Analysis of the Carcinogenic Activity of Certain Petroleum Products, A. M. A. Arch. Indust. Hyg. **4**:299, 1951.

34. Lorenz, E.: Radioactivity and Lung Cancer: A Critical Review of the Lung Cancer in the Miners of Schneeberg and Joachimsthal, J. Nat. Cancer Inst. **5**:1, 1944.

35. Haddow, A.: Mode of Action of Chemical Carcinogens, Brit. M. Bull. **4**:331, 1947.

36. Demerec, M.: Mutations Induced by Carcinogens, Brit. J. Cancer **2**:114, 1948.

37. (*a*) Berenblum, I.: The Mechanism of Carcinogenesis: Study of Significance of Cocarcinogenic Action and Related Phenomena, Cancer Res. **1**:807, 1941. (*b*) des Ligneris, M. J. A.: Precancer and Carcinogenesis, Am. J. Cancer **40**:1, 1940. (*c*) Friedewald, W. F., and Rous, P.: The Initiating and Promoting Elements in Tumor Production: Analysis of Effects of Tar, Benzpyrene, and Methylcholanthrene on Rabbit Skin, J. Exper. Med. **80**:101, 1944. Rusch and Kline.[11a]

cells to multipy to a mass of visible size, although this event accounts for part of the period. Thus, transplanted tumor cells grow out to a perceptible mass in a susceptible host in much less time than the usual latent period in tumorigenesis. Also, if carcinogen in the form of a pellet is withdrawn at weekly intervals after implantation in a series of animals, a point is found beyond which the irreversible changes have occurred in the surrounding cells and tumor appears in the absence of further stimulation.[38] Furthermore, if tissues enclosing a pellet of the carcinogen are transplanted, a point is found after some weeks at which the cells have become autonomous, as evidenced by their ability to multiply quickly into a perceptible tumor.[39]

An induction period is also found in human carcinogenesis. It conforms to the experimental facts as far as they are known, leading to the inference that it might so conform in the remainder of tumors. The occasional human tumors that are induced by physical carcinogens (radium, thorium, x-rays, solar radiation) or by chemical carcinogens (β-naphthylamine, benzene, tar, soot, petroleum products, chromates, arsenic) make their appearance only after an interval of several to many years.

It is this long induction time together with the small amount of required carcinogen which makes it so difficult to find additional human carcinogens and to prove a cause-and-effect relationship between exposure to a substance and a subsequent tumor. The presence of the carcinogens need not be continuous, and they do not multiply, unlike the agents in infectious diseases. The tumor may appear many years after cessation of the exposure, as in bladder dye-induced tumors, making it difficult or even impossible to recognize, recover, identify, and fulfill Koch-like postulates with the carcinogenic chemical. Only in the case of very persistent carcinogens, such as, for example, radium deposited in bone, would it be possible to recover the agent that had caused a tumor, inject it into animals and produce a new tumor, and then again recover the agent. There is evidence that some carcinogens are altered in the body. Successful recovery in such cases might merely represent the unutilized surplus and not that portion which had initiated the tumor.

Experimental carcinogenesis has demonstrated that the cells of a young animal can be converted to cancer cells about as easily as those of an old animal.[40] If the strength of a carcinogen is low in quantity or quality, the tumors appear later in life; the induction time is long. These two observations jointly may explain the age incidence of human cancer and supplant the older idea that there is something about the aging process itself which predisposes or leads to cancer. The great increase in incidence of human cancer with advancing age probably indicates a

38. Andervont, H. B., and Shear, M. J.: Production of Tumors in Mice Following Removal of Methylcholanthrene-Cholesterol Pellets, J. Nat. Cancer Inst. **2**:333, 1942.

39. Stewart, H. L.: Study of the Histological Changes and Transplantation of Tissue Surrounding Methylcholanthrene Pellets During the Latent Period of Tumor Development in Female C3H Mice, Am. J. Path. **15**:707, 1939.

40. Brunschwig, A., and Tschetter, D.: Age Factor and Latent Period in Production of Sarcoma by Methylcholanthrene in Rats, Proc. Soc. Exper. Biol. & Med. **36**:439, 1937.

long induction time due to low intensity and potency of the etiological agents.[41] If this interpretation is true, the difficulties in identifying the causes of the cancers are greatly increased. The curves for most types of human cancer resemble those in laboratory experiments when the agent is of low potency and small quantity. There is one view which holds that carcinogenic agents are merely accelefators, speeding up processes which would occur anyway. This concept might explain the shorter induction time, but not the greater number of tumors. It is hard, for example, to conceive of the preneoplastic nuclear changes induced by ionizing radiations leading to a greater number of tumors in x-ray technicians as merely an acceleration of a phenomenon which would have occurred without the exposures.

The significance of the induction time or latent period in human carcinogenesis appears great. Experimental observations show that during this time the cell, while morphologically relatively normal, is undergoing some profound change which may eventuate, when a certain threshold effect is reached, in a cell having new properties which characterize neoplasia. The treatment may be interrupted and then resumed, with only slight recovery of cells during the nontreatment period.[42]

If this concept applies also to man, then probably most persons have cells in one place or another which are part way through carcinogenic transformation and require only further carcinogenic stimulation to add up to an effective threshold dose. For example, if ultraviolet radiation of proper dose and wave length induces skin cancer, as appears certain from experimental work[43] and probably from statistical work in relation to man,[44] then it may be presumed that no human skins exposed to solar radiation are normal in this regard and that they are all part way through an effective induction. Such tissues might attain threshold carcinogenic dose levels with emergence of tumor from summation effects with a carcinogen of a different type, from co-carcinogenic action with a noncarcinogen (both to be described in subsequent sections), or from further exposure to the original agent.

Amount of Carcinogen and Percentage Yield.—In experimental carcinogenesis with a heavy dose of carcinogen, tumor develops in 100% of the animals.[45] The dose can be reduced to produce tumors in any desired percentage. The effective amount of a potent agent is very small. For example, an injection of 0.02 mg. of 20-methylcholanthrene per mouse produces sarcoma in over 50% of mice, and a few tumors can be induced with much less.[46] With small amounts of carcinogen, the percentage yield is reduced and the induction time is lengthened. On further

41. Kennaway, E. L., and Kennaway, N. M.: Relation Between the Incidence and Incubation Period of Cancer in Man, Yale J. Biol. & Med. **17**:139, 1944.

42. Rusch, H. P., and Kline, B. E.: Influence of Interrupted Carcinogenic Treatment on Tumor Formation, Proc. Soc. Exper. Biol. & Med. **69**:90, 1948.

43. Blum, H. F.: Sunlight and Cancer of the Skin, J. Nat. Cancer Inst. **1**:397, 1940. Rusch, H. P.; Kline, B. E., and Baumann, C. A.: Carcinogenesis by Ultraviolet Rays with Reference to Wavelength and Energy, Arch. Path. **31**:135, 1941.

44. Blum, H. F.: Sunlight as a Causal Factor in Cancer of the Skin in Man, J. Nat. Cancer Inst. **9**:247, 1948. Peller, S.: Epidemiology of Skin Cancer, J. Invest. Dermat. **11**:73, 1948.

45. Tannenbaum, A., and Silverstone, H.: Dosage of Carcinogen as a Modifying Factor in Evaluating Experimental Procedures Expected to Influence Formation of Skin Tumors, Cancer Res. **7**:567, 1947.

46. Greenstein, J. P.: Biochemistry of Cancer, New York, Academic Press, Inc., 1947.

reductions of dosage, a threshold level is reached and finally a subthreshold situation. In some instances the latter might yield some tumors if the life span were longer.

There is some evidence that the same principles hold in man. In no instance reported to date has the human attack frequency been 100%. In one generator gas works in Japan, where exposure to tars appears to have been heavy, 27 cases of lung cancer occurred in a comparatively small exposed population.[47] In most instances tumor develops in a comparatively small proportion of those exposed, indicating that either the amount or the potency of the carcinogen was low.

The threshold or subthreshold agents and doses, alone or in combinations, so well known in the laboratory, provide interesting and perhaps important points for consideration in regard to the human problem. To how many carcinogens in the various categories is man exposed? Is their effect eventually cumulative to effective levels? The low percentage yield makes demonstration of a suspicious agent by statistical analysis uncertain, and the low concentration of agent makes its chemical recovery from cancers or the exposed tissue difficult. Nevertheless, the problem appears to be an important one, which must be attacked.

Specificity of Carcinogenic Agents.—Most of the carcinogens are nonspecific, inducing neoplasms of many kinds according to the type of tissue exposed. Thus the polycyclic aromatic hydrocarbons induce sarcomas of many types, including osteosarcoma, fibrosarcoma, liposarcoma, rhabdomyosarcoma, and lymphosarcoma; carcinomas in many organs, including the stomach, intestine, lung, skin, and prostate; leukemia; intracranial tumors, and others. Since these compounds are poorly soluble in body fluids, they usually induce tumors at the point of first contact. Other classes of compounds, usually containing nitrogen in the molecule, are frequently transported within the body during metabolism, storage, or excretion. These compounds tend to produce specific types of neoplasms located at the points to which they are carried. β-Naphthylamine, causing tumors in the urinary tract of dogs or man, illustrates this class of compound. A few agents are versatile, causing tumors at many sites; 2-acetylaminofluorene, which is reported to initiate tumors in the liver, acoustic duct, thyroid, blood cells, mammary gland, bladder, stomach, and uterus of rats and mice, is an example of this type of agent.[48]

So far as present knowledge goes, carcinogen-induced tumors follow the same rules in human beings as in experimental animals. Some of the agents (e. g., tar, soot) cause new growths at the portal of contact; others (e. g., benzol, radium, thorium) act at the places of metabolism or storage; still others (e. g., β-naphthylamine) induce tumors at points of excretion. A few, such as arsenic, act widely. Some agents are specific for one type of tumor, and others are more versatile. Thus, radium has induced sarcoma of bone, carcinoma of the pharynx, fibrosarcoma, synovioma, and so on.

Knowing these facts, the alert observer seeking additional human carcinogens in specific instances can have a fair idea of the class of compounds most likely to be found.

---

47. Kawahata, K.: Über die berufliche Entstehung des Lungenkrebses bei der Generatorgas-Fabrikation, Gann **30**:341, 1936.

48. Bielschowsky, F.: The Carcinogenic Action of 2-Acetylaminofluorene and Related Compounds, Brit. M. Bull. **4**:382, 1947.

Potency of Carcinogen.—The known carcinogens exhibit a range of potency in constant dosage from high (100% yield) down through moderate to mild, marginal, and even subthreshold effects. The causes for these differences in activity are not known, although a number of theories have been suggested.[49] Some chemical compounds may be either marginal or submarginal in activity, depending on the conditions of testing. The degree of sensitivity of the substrate—the cell—must also always be considered in the equation.

From the small amount of information available for man, it appears that the potency of carcinogens varies for this genus also. Since the human race is apparently heterozygous with respect to susceptibility or resistance to tumor induction, the results from exposure to a carcinogenic hazard would be expected to be less uniform than those for an inbred, genetically homozygous strain of animals. Because a few men might be exceptionally sensitive, they might respond to submarginal agents which would be harmless to the majority. There is some evidence from occupational studies that it is not always the most heavily exposed person in whom tumor develops. These considerations suggest that such mild agents would be difficult to identify by either statistical or experimental methods. Nevertheless, this area of investigation appears to have important if not promising research possibilities.

Addition or Summation of Carcinogenic Effects.—Two or more different carcinogens may add or summate their effects to produce a tumor. This principle is thoroughly established in experimental carcinogenesis, although its limits are not yet known. The results depend in part on whether the agents are administered synchronously or metachronously, on whether they act at the same or at different sites, and on other considerations.

The factors—genetical, nutritional, hormonal, and so on—that govern the degree of susceptibility of the cells to neoplastic conversion are not regarded as additive carcinogens. In the laboratory, Hieger showed in 1936[50] that one chemical compound could complete the carcinogenic effects started by another. This observation was confirmed and extended by others,[51] in some instances with regard to agents of dissimilar types. Thus, tumor-inducing chemicals were additive to ultraviolet radiation[52] and to virus.[53] The principle applied also to leukemogenesis.[54]

---

49. Pullman, A.: Structure électronique et pouvoir cancérigène des hydrocarbures aromatiques condensés, Bull. Assoc. franc. étude cancer **33**:120, 1946. Greenwood, H. H.: The Reactivity of Aromatic Bonds, with Reference to Carcinogenic Compounds of 1:2-Benzanthracene, Brit. J. Cancer **5**:441, 1951. Iversen, S.: A Possible Correlation Between Absorption Spectra and Carcinogenicity, Copenhagen, Ejnar Munksgaards Forlag, 1949. Haddow.[35]

50. Hieger, I.: On the Mechanism of Carcinogenesis by Chemical Compounds, Am. J. Cancer **28**:522, 1936.

51. (*a*) Rusch, H. P.; Kline, B. E., and Baumann, C. A.: The Nonadditive Effect of Ultraviolet Light and Other Carcinogenic Procedures, Cancer Res. **2**:183, 1942. (*b*) Lavik, P. S.; Moore, P. R.; Rusch, H. P., and Baumann, C. A.: Some Additive Effects of Carcinogenic Hydrocarbons, ibid. **2**:189, 1942. (*c*) Stasney, J.; Paschkis, K. E.; Cantarow, A., and Rothenberg, M. S.: Neoplasms in Rats with 2-Acetaminofluorene and Sex Hormones, ibid. **7**:356, 1947.

52. Findlay, G. M.: Ultraviolet-Light and Skin Cancer, Lancet **2**:1070, 1928.

53. Rogers, S., and Rous, P.: Joint Action of a Chemical Carcinogen and a Neoplastic Virus to Induce Cancer in Rabbits: Results of Exposing Epidermal Cells to Carcinogenic Hydrocarbon at Time of Infection with Shope Papilloma Virus, J. Exper. Med. **93**:459, 1951.

54. Mixer, H. W., and Kirschbaum, A.: Additive Effects of X-Rays and Methylcholanthrene in Inducing Mouse Leukemia, Radiology **50**:476, 1948.

However, all carcinogens do not summate. Thus, chemicals of two different types,[55] ultraviolet irradiation and chemical carcinogen or Shope virus,[51a] and x-rays plus Shope virus [51a] did not summate under other conditions.

In man, no unequivocal examples of summation effects are known, although many are suspected. For example, no single cause can be demonstrated for oral cancers; perhaps they are due to a summation of the several factors now under suspicion. Indeed, this principle promises, more than any other except co-carcinogenesis, to explain the origin of some human tumors. If the assumption proves true, the causative agents will be hard to identify. If several different agents acting synchronously, serially, or intermittently in small amounts over an extended period together induce tumors, they will be difficult to recognize and prove because each may be present in subthreshold quantities, and they may no longer be present when the search is made.

The possible medicolegal implications of the principle of summation, or additive carcinogen, will be discussed in the section on co-carcinogenesis, since the subjects are closely related.

Co-Carcinogenesis.—The tumor-producing influence of a carcinogen may be augmented by that of a noncarcinogen; this effect is known as co-carcinogenesis. Thus an agent which is unable to induce tumors by itself may enhance or complete the action of a carcinogen, even if the latter is used in subthreshold doses. First demonstrated by Shear,[56] who found that one chemical (a noncarcinogenic fraction of creosote oil) could enhance the activity of another (3,4-benzpyrene), this principle has since been found to apply to other combinations, including a chemical carcinogen followed by a noncarcinogenic chemical;[57] a carcinogenic chemical plus trauma,[58] and a carcinogenic chemical plus gamma radiation,[59] beta radiation,[60] or

55. Jaffé, W. G.: The Response of Mice to the Simultaneous Application of 2 Different Carcinogenic Agents, Cancer Res. **7**:529, 1947.

56. Shear, M. J.: Studies in Carcinogenesis: Methyl Derivatives of 1:2-Benzanthracene, Am. J. Cancer **33**:499, 1938.

57. (*a*) Twort, J. M., and Twort, C. C.: Comparative Activity of Some Carcinogenic Hydrocarbons, Am. J. Cancer **35**:80, 1939. (*b*) Shubik, P.: Studies on the Promoting Phase in the Stages of Carcinogenesis in Mice, Rats, Rabbits, and Guinea Pigs, Cancer Res. **10**:13, 1950. (*c*) Sall, R. D., and Shear, M. J.: Studies in Carcinogenesis: Effect of the Basic Fraction of Creosote Oil on the Production of Tumors in Mice by Chemical Carcinogens, J. Nat. Cancer Inst. **1**:45, 1940. (*d*) Morton, J. J., and Mider, G. B.: Effect of Petroleum Ether Extract of Mouse Carcasses on Skin Tumor Production in C57 Black Mice, Pub. Health Rep. **55**:670, 1940. (*e*) Kreyberg, L.: Influence of Dinitrokresol on the Development of Tar Tumors in Mice, Am. J. Cancer **36**:51, 1939. (*f*) Berenblum, I., and Shubik, P.: The Role of Croton Oil Applications, Associated with a Single Painting of a Carcinogen, in Tumor Induction of the Mouse's Skin, Brit. J. Cancer **1**:379, 1947. (*g*) Klein, M.: The Action of Croton Oil in the Induction of Sarcomas in Mice, J. Nat. Cancer Inst. **11**:843, 1951.

58. Linell, F.: On the Tumour-Promoting Effect of a Single Mechanical Trauma; Experimental Study on Skin Tumours in Tarred Rabbits, Acta path. et microbiol. scandinav., Supp. 71, p. 1, 1947. Berenblum, I.: Further Investigations on the Induction of Tumours with Carbon Dioxide Snow, Brit. J. Exper. Path. **11**:208, 1930. Pullinger, B. D.: An Experimental Approach to the Problem of Trauma and Tumours, J. Path. & Bact. **57**:467, 1945. Riley, J. F., and Pettigrew, F. W.: Acceleration by Means of Prolonged Mechanical Irritation of Carcinogenesis in the Skin of Mice Painted with 1:2:5:6-Dibenzanthracene, Brit. J. Exper. Path. **26**:63, 1945. MacKenzie, I., and Rous, P.: The Experimental Disclosure of Latent Neoplastic Changes in Tarred Skin, J. Exper. Med. **73**:391, 1941. Shubik.[57b]

(Footnotes continued on next page)

visible light.[61] Combinations of stimuli found not to be co-carcinogenic include methylcholanthrene plus heat administered concurrently [37b] and a chemical carcinogen plus any one of many chemical irritants.[57b] Much more work is needed to determine the limits and potentialities of this principle.

The simple difference between summation of carcinogens and the action of co-carcinogens is that the former is concerned with the additive effects of two or more active agents, while the latter is concerned with the enhancement of the effects of an active agent by the action of a noncarcinogenic agent. This distinction, however, is sometimes obscure, because there are many mild, marginal, subthreshold, and conditional agents whose carcinogenicity has not been recognized and which are therefore mistaken for co-carcinogens. In a given experience it is sometimes difficult to distinguish between these actions, but the distinction ought to be maintained, whenever possible, for the present.

No proved examples of co-carcinogenesis in man come to mind, although many situations suggest that this principle is in operation. The effects of trauma on skin previously prepared or altered by ultraviolet irradiation might, conceivably, cause a cancer where either agent alone would not. In the uterine cervix, for which expectancy of cancer increases with the number of pregnancies, the effects of hormonal stimulation might be co-carcinogenic when added to trauma and infection.

The possible medicolegal implications of co-carcinogens, of summation effects, and of synergism (to be described) appear great. For example, trauma is sometimes alleged to have caused a tumor. Because of failure to demonstrate experimentally that simple trauma induces neoplasms and because the events that precede the tumor rarely conform to criteria, especially with respect to length of induction time, which have been rather widely accepted, this factor is usually not regarded as the cause of a tumor. However, trauma is a co-carcinogen in certain experiments, and this possibility must be reconsidered in the case of some human cancers.

Synergism.—The combined carcinogenic effects of two agents may be greater than the sum of their individual effects. Thus, synergism has been reported in the induction of liver tumor by combinations of some azo dyes but not of other agents,[62] in leukemogenesis by x-rays and methylcholanthrene,[63] and by x-rays plus estrogenic hormones.[64] Synergism is here used in the same sense as in drug action. It ought to be kept separate from the concept of summation, or additive effects.

---

59. Mottram, J. C.: Production of Epithelial Tumours by Irradiation of a Precancerous Skin Lesion, Am. J. Cancer **30**:746, 1937.

60. Mottram, J. C.: Production of Epithelial Tumours by a Combination of Beta Radiation and Painting with Benzpyrene, Am. J. Cancer **32**:76, 1938.

61. Morton, J. J.; Mider, G. B.; Luce-Clausen, E. M., and Mahoney, E. B.: The Effect of Visible Light on the Development in Mice of Skin Tumors and Leukemia Induced by Carcinogens, Cancer Res. **11**:559, 1951.

62. MacDonald, J. C.; Miller, E. C.; Miller, J. A., and Rusch, H. P.: The Synergistic Action of Mixtures of Certain Hepatic Carcinogens, Cancer Res. **12**:50, 1952.

63. Furth, J., and Boon, M. C.: Enhancement of Leukemogenic Action of Methylcholanthrene by Pre-Irradiation with X-Rays, Science **98**:138, 1943.

64. Kirschbaum, A.; Shapiro, J. R., and Mixter, H. W.: Synergistic Action of Estrogenic Hormone and X-Rays in Inducing Thymic Lymphosarcoma in Mice, Proc. Soc. Exper. Biol. & Med. **72**:632, 1949.

No examples of synergism in human carcinogenesis have been reported, although they probably exist, to be recognized as such some day by an observer who is aware of the principle.

Inhibition of Carcinogenic Effects.—The carcinogenic effects of one carcinogen may be experimentally inhibited by those of another. Thus, Griffin, Brandt, and Setter [65] showed inhibition of azo-dye liver tumors by nitrogen mustard (methyl bis- or tris-[β-chloroethyl] amine hydrochloride, and Richardson, Stier, and Borsos-Nachtnebel [66] did the same with 20-methylcholanthrene. In these examples the agents were dissimilar in chemical type and possibly in primary site of action. Strong carcinogenic chemicals were partly inhibited by weak ones in epidermal carcinogenesis by Hill and associates,[67] by Lacassagne, Buu-Hoi, and Rudali,[68] and by Crabtree.[69]

There is at the present time no equivalent observation on human carcinogenesis. From analogy with experimental work and with drug actions in general, there should be. Inhibition effects will be extremely difficult to prove in man because the end-point is an absence of something (tumor) in populations where the great majority of persons normally show this absence.

Anticarcinogenesis.—The carcinogenic effects of a carcinogen may be inhibited by those of a noncarcinogen administered as an added factor in treatment. This effect may be observed as a lessening of the tumor yield or a lengthening of the induction time. The general factors, such as genetical, nutritional, hormonal, and metabolic status, which so greatly affect tumor response, are excluded from this category, as are those related to the vehicle or solvent, themselves factors of considerable importance.[70] Anticarcinogenesis is distinguished from inhibition on the basis that the second agent in anticarcinogenesis is itself not carcinogenic. However, this distinction is not always sharp. In experimental carcinogenesis the phenomenon is well documented. Thus, benzpyrene carcinogenesis has been decreased on skin by bromobenzene [70] and in connective tissues by glyceraldehyde.[71] Estradiol-induced fibromas have been inhibited by progesterone.[72]

No examples of anticarcinogenesis in man are known, although they probably exist, and the principle may be an important one. Falk and I recently reported

65. Griffin, A. C.; Brandt, E. L., and Setter, V.: Nitrogen Mustard Inhibition of Azo Dye Carcinogenesis, Cancer Res. **11**:868, 1951.

66. Richardson, H. L.; Stier, A. R., and Borsos-Nachtnebel, E.: Liver Tumor Inhibition and Adrenal Histologic Responses in Rats to Which 3'-Methyl-4-Dimethylaminoazobenzene and 20-Methylcholanthrene Were Simultaneously Administered, Cancer Res. **12**:356, 1952.

67. Hill, W. T.; Stanger, D. W.; Pizzo, A.; Riegel, B., and Wartman, W. B.: Inhibition of Skin Carcinogenesis in Mice by Mixtures of Strong Carcinogens, Cancer Res. **12**:270, 1952.

68. Lacassagne, A.; Buu-Hoi, and Rudali, G.: Inhibition of the Carcinogenic Action Produced by a Weakly Carcinogenic Hydrocarbon on a Highly Active Carcinogenic Hydrocarbon, Brit. J. Exper. Path. **26**:5, 1945.

69. Crabtree, H. G.: Anti-Carcinogenesis, Brit. M. Bull. **4**:345, 1947.

70. Dickens, F.: The Influence of the Solvent on the Carcinogenic Response, Brit. M. Bull. **4**:348, 1947.

71. Riley, J. F., and Pettigrew, F.: Retarding Effect of Glyceraldehyde on Benzpyrene Sarcoma Formation in Mice, Cancer Res. **4**:502, 1944.

72. Iglesias, R.; Lipschütz, A., and Nieto, G.: Antifibromatogenic Effects Produced by the Intermittent Action of Progesterone, Cancer Res. **4**:510, 1944.

that the carcinogen in soot, which was recognized in 1775 by Pott as inducing some human skin cancers and has frequently been shown since then to be present in extracts of soot, is indeed 3,4-benzpyrene, among other hydrocarbons.[30b] This compound is highly carcinogenic in animals. It has been identified in atmospheric dusts collected in Chicago [30b] and in eight English cities.[30a] Yet little if any correlation is observed between the amount of atmospheric soot and the incidence of human lung cancer. How is this apparent discrepancy explained? It may be an example of anticarcinogenesis. It is easy to demonstrate that carbon black, the chief component of soot, in finely divided form is an excellent adsorber of 3,4-benzpyrene.[73] In the adsorbed state, this carcinogen may be biologically inactive and incapable of exerting its usual carcinogenic effects. If this hypothesis proves to be correct, the action of soot may be an outstanding example of anticarcinogenesis in man. On the other hand, it may merely be an example of unavailability of carcinogen.

Epicarcinogenesis.—By this term is meant the transformation of a benign to a malignant tumor by the continued effects of a carcinogen or a co-carcinogen. This phenomenon is well grounded in experimental evidence, and there is probably a counterpart in man. Skin painting of animals by a number of chemicals results in benign epithelial growths which may sometimes be converted to carcinomas by continuing the applications,[74] by traumatizing the lesion,[75] or by causing a virus to localize in it.[76] In the human, trauma to a benign melanoma or trauma by incomplete removal of a bladder or laryngeal papilloma may hasten its conversion to a malignant neoplasm. There is scattered observational evidence that benign adenomatous polyps of the colon may later be polypoid carcinomas. One naturally wonders whether the change indicates that a carcinogen has caused the transformation and, if so, what it may be.

The sequence from normal to benign to malignant cell indicates a step-like series rather than a single change. If the fundamental process is mutation, this supposedly rare event would have to occur sequentially several times, rather than only once, and always in the direction of greater malignancy. Intermediate changes during mutation have been described,[77] but these changes are not in themselves mutants. Mottram [74a] clearly showed that chemical carcinogen can indeed change normal to benign tumor cells and these in turn to malignant cells.

Conditional Neoplasia.—Certain proliferative lesions have been observed in experimental carcinogenesis which have all the morphological and some of the biological characteristics of cancer, including invasiveness and even metastases; yet they are not autonomous, so that they regress if the inducing agent is withdrawn. They are neoplasms only under special conditions, and so they are designated

73. Falk, H. L., and Steiner, P. E.: The Adsorption of 3,4-Benzpyrene and Pyrene by Carbon Blacks, Cancer Res. **12**:40, 1952.

74. (*a*) Mottram, J. C.: The Change from Benign to Malignant in Chemically Induced Warts in Mice, Brit. J. Exper. Path. **26**:1, 1945. (*b*) des Ligneris.[37b]

75. des Ligneris [37b] and others.

76. Rous, P., and Kidd, J. G.: The Carcinogenic Effect of a Papilloma Virus on the Tarred Skin of Rabbits: Description of the Phenomenon, J. Exper. Med. **67**:399, 1938.

77. McElroy, W. D.: Evidence for the Occurrence of Intermediates During Mutation, Science **115**:623, 1952.

conditional neoplasms. Described by Rous and Kidd [78] in relation to certain tar-induced tumors in rabbits, the concept has since had wider recognition.[79]

No exact equivalent in man of the experimental observations is known although three examples come close. Removal of a primary cancer is alleged to have caused regression of established metastases in a few cases.[80] A better example may be regression of the cells of carcinoma of the prostate if the hormone support on which they are in part dependent is withdrawn by orchiectomy. Other examples of analogous hormone dependence can be recalled. These examples differ from the experimental in that the human tumors arise from cells which in the normal state are dependent on the agent which is withdrawn. Finally, there are the reports of regression of bladder tumors if the urinary stream is diverted.[81]

It is probable that human analogies to the experimental observations exist and that they will be recognized and reported. The usefulness of this concept in therapy offers promise which remains to be explored.

Carcinogenic Importance of the Events in Early Life.—Scattered data have been accumulating in recent years which suggest that the events during early life are extraordinarily important in determining the appearance of tumor later in life. There are indications that exposure to carcinogenic stimuli early in life may lead to cancer many years later and, furthermore, that a tumor-inducing stimulus may be more effective at an early age than an equal exposure later in life. Present experimental evidence on the latter point is equivocal, but there is support for the former, although not yet to the extreme degree suggested by observations on man.

For example, there is evidence that circumcision at an early age, according to the Jewish rite, fully protects against penile cancer but that the same operation after the age of 5 years, as in the Mohammedan practice, does not.[82] If this observation is correct, the fact must be accepted that in some persons a chain of events is set up before the fifth year of life which eventuates in cancer 30 to 60 years later.

There is also evidence that persons who are born and reared in parts of the world where the frequency of primary carcinoma of the liver is high retain a tendency toward development of this tumor later in life even if they move at an early age into a geographical area where prevalence of liver cancer is low. The Chinese immigrant in Vancouver, B. C., Canada,[83] and the Filipino in Los Angeles [84] exhibit the high frequency of liver cancer of their country of origin. Berman [25] found that

78. Rous, P., and Kidd, J. G.: Conditional Neoplasms and Subthreshold Neoplastic States: A Study of Tar Tumors of Rabbits, J. Exper. Med. **73**:365, 1941.

79. Flory, C. M.: The Production of Tumors by Tobacco Tars, Cancer Res. **1**:262, 1941.

80. Mann, L. T.: Spontaneous Disappearance of Pulmonary Metastases After Nephrectomy for Hypernephroma: Four Year Follow-Up, J. Urol. **59**:564, 1948. Rhodenburg, G. L.: Fluctuations in the Growth Energy of Malignant Tumors in Man, with Special Reference to Spontaneous Cures, J. Cancer Res. **3**:193, 1918.

81. Davis, E.: Disappearance of Carcinomatous Ulceration of Bladder Following Ureterosigmoidostomy: Report of 2 Cases, J. A. M. A. **137**:450, 1948.

82. Kennaway, E. L.: Cancer of the Penis and Circumcision in Relation to the Incubation Period of Cancer, Brit. J. Cancer **1**:335, 1947.

83. Strong, G. F.; Pitts, H. H., and McPhee, J. G.: Primary Carcinoma of the Liver, Ann. Int. Med. **30**:791, 1949.

84. Steiner, P. E.: Unpublished data.

the Portuguese East African Bantu laborer working in the Rand gold mines had a frequency of liver cancer six times that of the native South African Bantu. In these examples, the possibility cannot be excluded that the hepatomagenic factor emigrated also and continued its action in the new environment. In any event, the principle is clearly demonstrated.

Some evidence has accumulated that heavy smoking before the age of 25 years may be more important in the etiology of lung cancer than heavy smoking begun later in life [85] and that early marriage and child bearing are predisposing factors to uterine cancer.

It is customary to recognize the great importance of events of early life in nutrition, anatomy, psychology, religion, and other areas, but to include cancer in this category requires readjustment in our thinking.

Carcinoma-in-Situ.—This term has come into usage in recent years to characterize minute proliferative lesions in various epitheliums which are believed to be carcinomas although they have not yet infiltrated into the adjacent supporting connective tissue. The term may mean either a precancerous lesion which is inevitably destined to become invasive cancer or the preinvasive stage of true cancer.

This concept has arisen as a result of the laudable efforts to discover the earliest stages of cancer, if not precancer.

That such morphological lesions exist in many epithelial surfaces and glands is beyond doubt, but that they are invariably cancer is not yet fully proved. This point is difficult to establish for two reasons. In diagnosing the lesion microscopically, it may be entirely removed, leaving no cells to confirm or deny the concept by their subsequent behavior. These lesions may be quiescent for long periods, even years, so that their course is difficult to follow.

Some doubt is cast on the validity of the concept by failure of experimental confirmation. Although the cellular changes in experimental carcinogenesis have been carefully studied in much readily available suitable material, no lesion is recognized analogous to what is currently called carcinoma-in-situ in the human. The long induction periods during which the cells are being biased to cancer are not characterized morphologically by "carcinoma-in-situ," and when the cells do change to what is morphologically characteristic of cancer, they do not remain indolent for long periods but grow out quickly.

Every carcinoma must have microscopic beginnings during which it is preinvasive, but the concept of a long, almost quiescent, indolent stage subsequent to morphological anaplasia but prior to the rapid growth and invasiveness characteristic of malignant tumors has no known experimental counterpart, and much more study of this lesion in man is desirable.

Precancer.—This term is, unfortunately, being widely used to characterize two different concepts, to the confusion of both. According to one usage precancer means a histological lesion composed of altered cells which do not yet exhibit invasive tendencies but which are known on the basis of past experience with similar lesions to be inevitably cancerous. According to the second usage this term refers to lesions

85. Doll, R., and Hill, A. B.: Smoking and Carcinoma of the Lung: Preliminary Report, Brit. M. J. **2**:739, 1950.

composed of altered cells which are known on a statistical basis to give rise to cancer with significant frequency. The practicing pathologist uses this word in the former sense; the experimental oncologist, frequently in the latter.

Pathologists are aware of the hazards of calling any lesion precancerous unless they have previously followed the course of similar lesions. This conservatism has had experimental justification by Aronson, who observed in guinea pigs skin changes which resembled precancerous lesions three weeks after the application of 1:2:5:6-dibenzanthracene was begun and yet did not go on to cancer during five more years of daily treatment with the carcinogen. In clinical work, in which the diagnosis is used as a guide in treatment, the most precise usage of the term precancer is needed. The term should be used to designate only those lesions which inevitably will become cancer. In the experimental laboratory, where human life is not at stake, the broader but looser concept is useful.

SUMMARY

Some of the recent observations in experimental carcinogenesis have a recognized human counterpart. Many more probably have an unrecognized equivalent in man, accounting for the origin of some tumors now regarded as spontaneous. Knowing some of the basic principles of carcinogenesis, the alert physician should be able to recognize additional examples of cause-and-effect relationships. Some relations of the nature, amount, potency, and specificity of carcinogens to induction time and percentage yield have here been discussed and correlated with the principles of summation effects, co-carcinogenesis, synergism, inhibition, anticarcinogenesis, and epicarcinogenesis. Precancer, carcinoma-in-situ, conditional neoplasia, and the carcinogenic importance of the events of early life are briefly interpreted in the light of these concepts.

Laboratory Methods and Technical Notes

VERTEBRAL COLUMN

Methods for Removal, Reconstruction, and Gross Sectioning

GOLDEN SELIN, M.D.
SAMUEL SCHLYEN, M.D.
AND
HENRY L. JAFFE, M.D.
NEW YORK

IN RECENT times probably no pathologist has done more than Schmorl to direct attention to the pathologic anatomy of the vertebral column. In the course of a number of years of concentrated interest in problems relating to the vertebral column, many thousands of columns were removed at his institute, in Dresden. The method of removal used at that institute was to expose the vertebral column through an incision in the back, similar to that commonly used for removal of the spinal cord. The column was then disconnected by saw cuts from the ribs and pelvic girdle, and the cervical end was disconnected from the skull either by making a transverse saw cut through the column or by chiseling around the foramen magnum.[1] Others have used different methods for removing the column or studying it in situ. Certainly in the latter connection the method described by Schmincke [2] is too complex to be justifiable in view of the ease with which one can actually remove the column and reconstruct the body.

Neither in Schmorl's [1] nor in Beadle's [3] account of the technique practiced in Dresden nor in such standard manuals on autopsy technique as that by Farber [4] does one find directions for reconstruction of the body after removal of the vertebral column or some segment of it.

It has seemed to us that the examination of an ample segment of the vertebral column as part of the routine of a postmortem examination would be a commoner practice if the removal were done through the usual ventral autopsy incision and the continuity of the body restored to the satisfaction of all interested parties by an easily assembled reconstruction. Therefore, we shall describe our routine for removing the columnar segment extending from the fourth or fifth thoracic through the fifth lumbar vertebra, give concrete directions for reconstruction of the body, and add some suggestions for sectioning which facilitate examination of the extirpated specimen. In connection with some special study the technique which we shall describe can be applied, with appropriate modifications, to the removal of the entire vertebral column or some other specific area, such as the lumbosacral, including the sacroiliac articulations, but we shall not deal here with such modifications.

From the Laboratory Division, Hospital for Joint Diseases.

This work was aided by a grant from the Henry Foundation, Inc., New York.

1. Schmorl, G.: Zur Sektionstechnik der Wirbelsäule, Zentralbl. allg. Path. **48**:7, 1930.

2. Schmincke, A.: Zur Sektionstechnik der Wirbelsäule, Zentralbl. allg. Path. **47**:177, 1929.

3. Beadle, O. A.: The Intervertebral Discs: Observations on Their Normal and Morbid Anatomy in Relation to Certain Spinal Deformities, Medical Research Council, Special Report Series, No. 161, London, His Majesty's Stationery Office, 1931.

4. Farber, S.: The Postmortem Examination, Springfield, Ill., Charles C Thomas, Publisher, 1937.

EQUIPMENT

Instruments and Supplies.—In addition to a band saw, the instruments needed are merely: a 6-in. (15-cm.) "boning knife"; two "parquet floor chisels," one of which is 1½ in. (3.8 cm.) and the other 3 in. (7.6 cm.) in width; a standard nail hammer; and two towel-clip forceps such

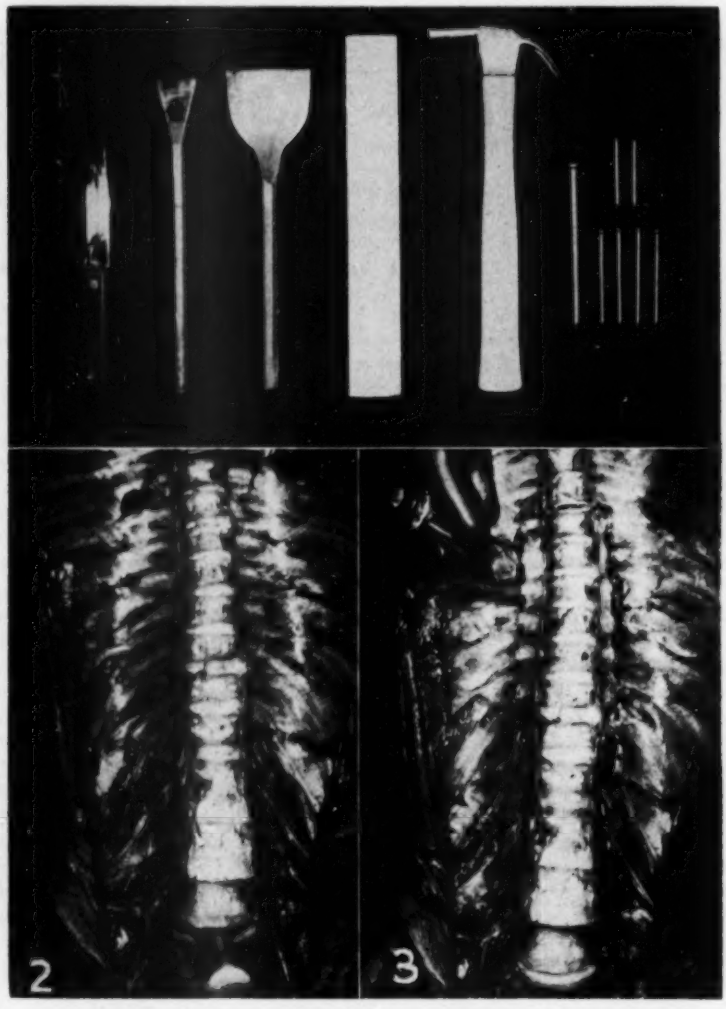


Fig. 1.—Some of the needed instruments and supplies.

Fig. 2.—Chisels inserted into intervertebral disks in thoracic and lumbosacral areas as first step in removal of vertebral segment.

Fig. 3.—Severance of the ribs, close to each side of the vertebral segment.

as are ordinarily used in a hospital operating room. The supplies necessary for each spine reconstruction consist of about 2 ft. (61 cm.) of pine or hemlock "2 in. square stock"; one "60 d" spike; six "10 d" nails; about 4 ft. (122 cm.) of plumber's oakum, and about 2 lb. (0.9 kg.) of plaster of Paris (Fig. 1).

Band Saw.—A band saw is an essential part of the equipment of any pathology laboratory in which bone is examined. To be properly versatile, a band saw used in a laboratory not only should be able to make straight as well as curved cuts through bone and wood but should also meet the following specifications:

(*a*) Cut fresh, unfixed, and unfrozen bone of any consistency into slices a few millimeters in thickness without fragmentation; (*b*) cut ivory-hard or eburnated bone without burning or carbonization; (*c*) cut skin, muscle, other soft tissues, and bone in a single operation without ripping or tearing; (*d*) cut tissues of any consistency, including intervertebral disc tissue, without binding or appreciably retarding the speed of the blade, and (*e*) produce a minimum amount of bone dust without grinding the superficial removable dust into the deeper layers of the bone.

The inexpensive stock model Delta Homecraft 10-inch wood-cutting band saw (#28-110) is easily modified to meet these specifications, which mechanically require a slow blade speed, full range of cutting power, and a highly tempered, flexible, dust-removing blade.

For cutting bone, with or without the attached soft parts, we have found that the optimum blade speed is 1,200 board feet per minute. To obtain this speed, we have mounted a 7-in. (18 cm.) pulley on the saw shaft, and a 2-in. (5 cm.) pulley on the shaft of a motor having an estimated speed of 1,725 revolutions per minute (R.P.M.). Sufficient power to cut at an even, uninterrupted speed is delivered from a ⅓ horsepower direct-current motor or a ½ horsepower sixty-cycle alternating-current motor.

We have found that the blade requirements are particularly well fulfilled by using a metal-cutting saw blade that is ¼ in. (0.64 cm.) wide and has 6 teeth to the inch, every other tooth being absent or skipped. This blade is known to saw dealers as a "six tooth-skip tooth metal-cutting blade."

While the saw we have modified for our use is a 10-inch (25 cm.) wood-cutting Delta Homecraft band saw, any make and size of band saw can be modified to fulfill these requirements by computing the necessary pulley diameter, motor speed, and motor power changes from the manufacturer's listed specifications, using the Homecraft model as a standard.

REMOVAL OF SPINAL SEGMENT

After the viscera have been removed from the body through the ventral incision, the lumbar vertebral bodies and transverse processes are stripped of their muscular attachments. The spine is then severed transversely at the junction of the fifth lumbar and first sacral vertebrae and again at the junction of two of the upper thoracic vertebrae (Fig. 2). This is done by hammering the 3-in. chisel into the intervertebral disk. After a few well-delivered blows, the chisel completely divides the disk, intervertebral facet joints, and spinous processes. The ribs along both sides of the vertebral segment are severed at a distance of 1 in. (2.5 cm.) from the costovertebral articulations, with the smaller chisel and the hammer (Fig. 3). At this stage, each end of the vertebral segment is grasped with a towel forceps, and the incision line of the severed ribs is widened by pulling the segment upward and laterally. The boning knife is inserted into this enlarged chisel line, and the muscles and other soft tissues are severed by directing the blade dorsally and medially toward the spine throughout its entire length (Fig. 4). The knife cuts are repeated several times on each side until the segment can be rotated and dislocated from its bed. Finally, the detached portion of the spine is removed from the body by constantly elevating one of its ends while skinning the remaining thin soft tissues of the back from the tips of the transverse processes (Figs. 5 and 6).

RECONSTRUCTION OF THE COLUMN

The reconstruction of the vertebral column is achieved by restoring its continuity with a firmly anchored noncollapsible substitute. For this purpose, a length of 2-in. (5-cm.) square wood stock, 1½ in. (3.8 cm.) longer than the removed columnar segment, is used as the replacement. The ends of the wood stock are then mitered to parallel the slanted ends of the specimen. A 6-in. (15-cm.) (60 d) spike is hammered 2½ in. (6.4 cm.) into the upper, or thoracic, end of the square stock, and the head of the nail is then cut off with a hacksaw (Fig. 7).

The full length of the protruding nail is driven into the center of the bodies of the thoracic vertebral stump by hammering on the end of the square stock (Fig. 8). If there is a marked kyphosis of the thoracic stump, it is advisable to bend the nail to this contour to prevent a posterior perforation of the vertebrae and skin. When the end of the square stock lies flush against the thoracic vertebra, the nail becomes an anchored iron girder uniting the thoracic end of the

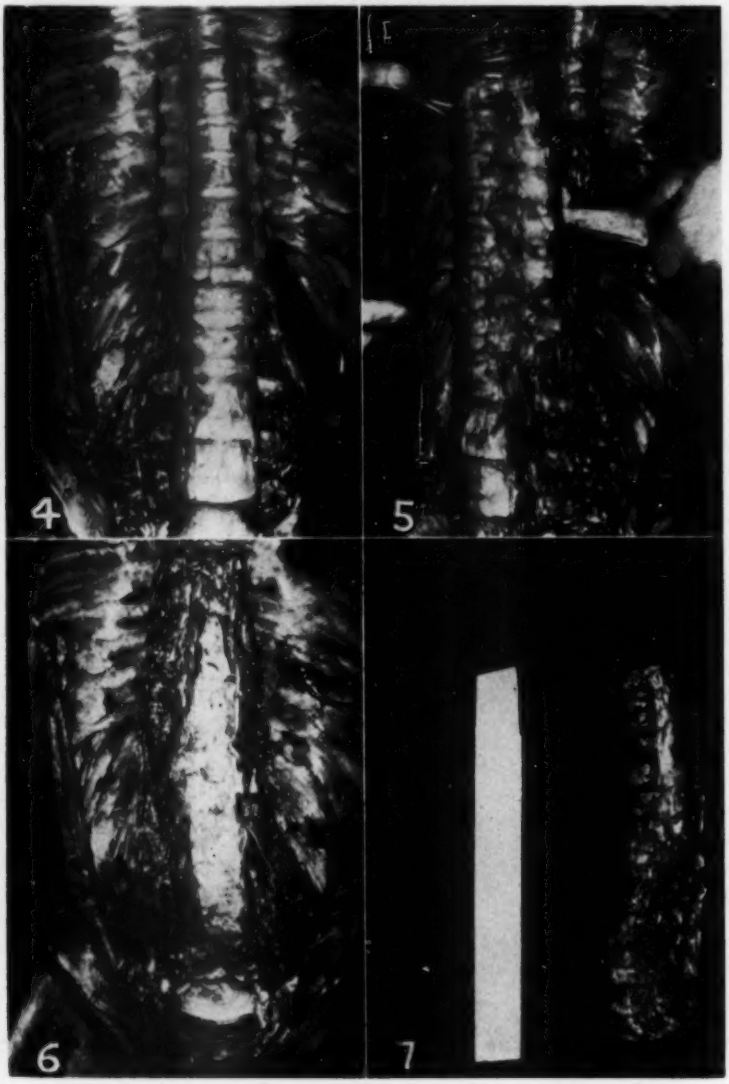


Fig. 4.—Widening and undercutting along the incision line through the ribs and the soft tissues along the lumbar vertebrae. The towel clips are not shown.

Fig. 5.—Spinal segment dislocated from its bed, being separated from adherent soft tissues posteriorly.

Fig. 6.—Appearance of body after removal of spinal segment.

Fig. 7.—Mitered wood stock with headless spike at upper end, next to the spinal segment which it is to replace.

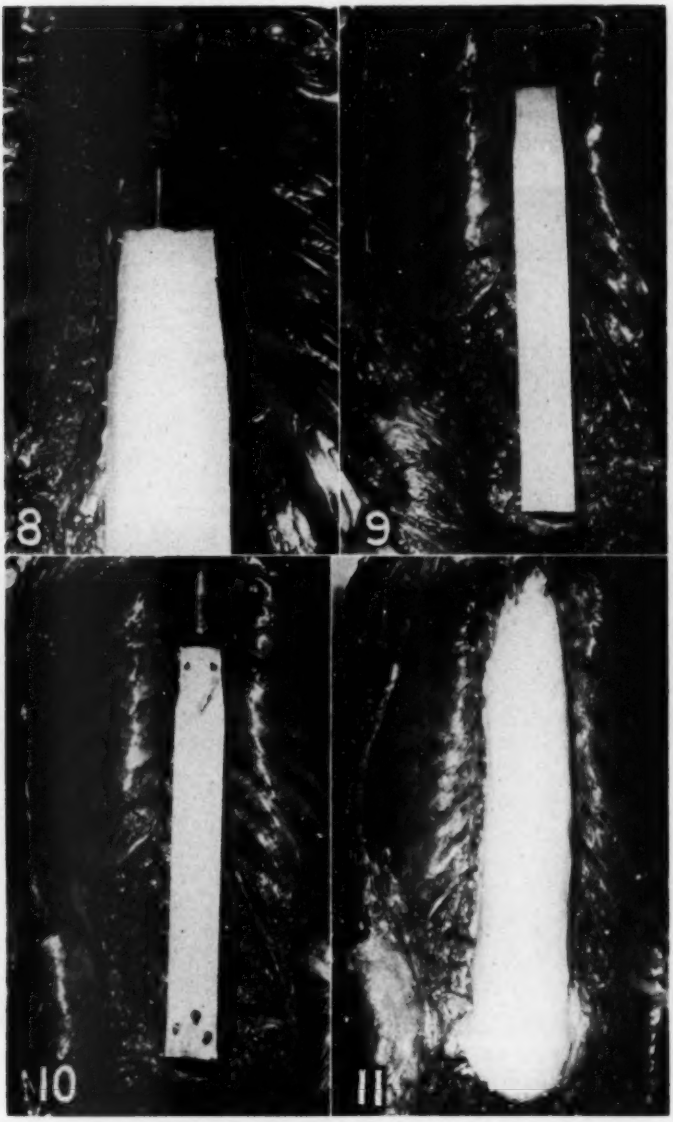


Fig. 8.—"Spiked" wood stock in process of being driven into bodies of thoracic vertebral stump.

Fig. 9.—Wood stock in final position.

Fig. 10.—Position of the six nails which are being driven in at a slant ("toe-nailed") to join the wood stock firmly to the sacral stump. The three nails being driven through the sacrum toward the stock are not too clearly visible. The two nails at the thoracic end of the stock, driven in to prevent rotation at that end, do stand out clearly.

Fig. 11.—Completed reconstruction of the column. Plaster-of-Paris has been molded around the wood stock, which was first covered with strands of oakum to make the plaster adhere better.

reconstruction. By gentle downward pressure and slight stretching of the intervening space, the square stock is made to lie snugly between the thoracic stump and the sacrum (Fig. 9).

The sacral end of the square stock is then firmly joined to the sacral stump by four or six 3-in. (10 d) nails. Rotation at the thoracic end is prevented by two 2-in. (6 d) nails driven through the wood into the vertebral column stump (Fig. 10).

If, as sometimes happens, the sacrum slants into the pelvic floor at a very acute angle, it is extremely difficult to hammer the nails into the wood stock from the sacral stump. When this

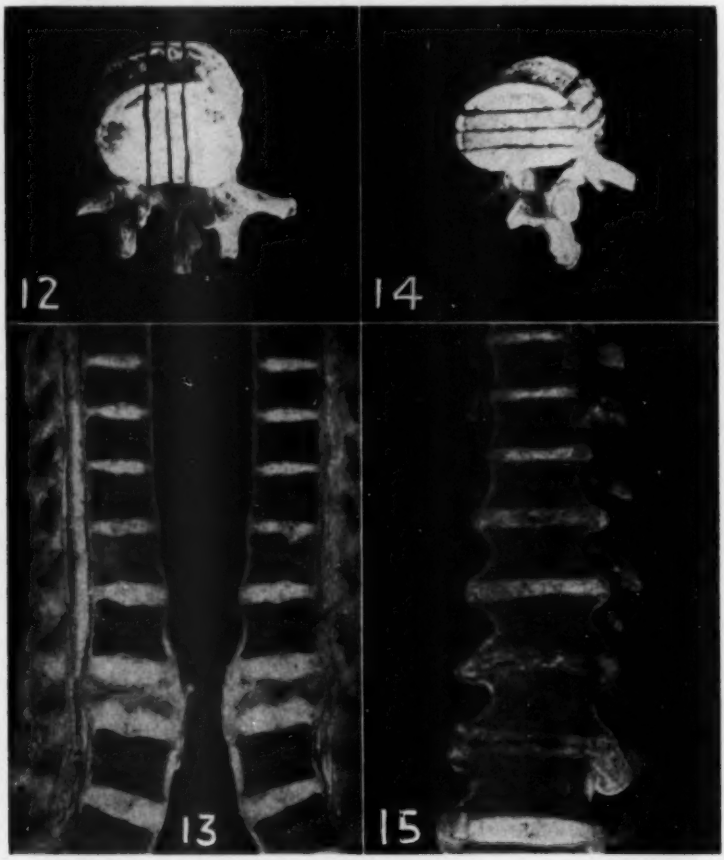


Fig. 12.—Diagram of lines for sectioning the columnar segment in the sagittal plane.

Fig. 13.—Columnar segment cut in sagittal plane, slightly off center. The spinal cord is seen intact in its setting. The first lumbar vertebra, which is involved by metastatic cancer, is collapsed and bulges toward the spinal canal. A focus of metastatic cancer can also be seen in the posterior half of the 11th thoracic body, but the contour of that body has remained unaffected. Additional abnormalities are also visible but need not be pointed out in detail.

Fig. 14.—Diagram of lines for sectioning the column in the frontal plane.

Fig. 15.—A columnar segment cut in the frontal plane. The cut reveals extensive degeneration of some of the intervertebral disks and pronounced exostosis formation.

situation occurs, the reconstruction at the sacral end may be modified as follows: A ¼-in. (0.64 cm.) hole is drilled through the body of the first sacral vertebra from its lumbar end. A 3 by ¼-in. lag screw with a washer at its head is turned through the hole in the sacrum from

the direction of its pelvic surface, into the square stock, by a wrench. When the sacral stump and the wood stock are tightly pulled together the union is further strengthened by toe-nailing two 10 d nails into the sacrum.

Before proceeding with the next step, one should test the firmness of the reconstruction by lifting the body a few inches from the table, using the square stock as a handle. The reconstruction is considered adequate when the united structures remain practically immovable. Thin strands of oakum are then wrapped around the square stock to make an adherent bed for a plaster-of-Paris covering, which is molded and shaped about wood to restore the continuity of the vertebral column (Fig. 11).

EXAMINATION OF REMOVED SPINAL SEGMENT

The obscuring soft tissues are cut away, and the segment of vertebral column is inspected and palpated for any abnormalities. If the cleaned, intact specimen is then x-rayed in the anteroposterior and lateral positions, one obtains detailed x-ray pictures unobscured by extraneous

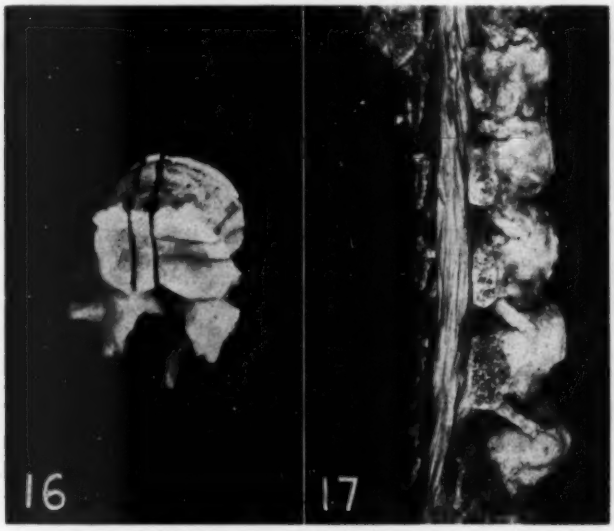


Fig. 16.—Diagram of lines for sectioning a column in both the sagittal and the frontal plane.

Fig. 17.—Spinal cord and nerves coming off from it shown up by unroofing the spinal canal and intervertebral canals. Note the spinal nerves coming through the unroofed intervertebral foramina.

shadows. If the x-ray pictures are used as a guide, the plane or planes in which the vertebral segment is sawed can be chosen in such a way that they will demonstrate the pathologic anatomy to its fullest extent. The choice of the plane or planes for cutting, and the extent to which the specimen is cut up, will depend, of course, upon the particular center of interest in the specimen or case. In cutting slices in one or more planes, a desirable thickness has been found to be 3/16 in. (0.5 cm.).

Whatever slices are cut are then washed in a stream of cold tap water and brushed gently with a soft handbrush to remove any remaining superficial bone dust. Pathologic alterations observed can then be photographed. If the photographs are to be made in color, they should be taken while the specimen is still fresh, since otherwise the intervertebral disk tissue becomes discolored by blood. If one wishes, x-ray films of the split specimen and its component slices can likewise be taken, but this can be done after the specimen has been fixed. X-ray pictures made of the sliced specimen are particularly useful if one wishes to correlate the microscopic findings with the general gross and roentgenographic findings.

As indicated, the plane or planes in which the removed columnar segment is sectioned will naturally be chosen in accordance with the purposes of the examination. Often one will want merely a general orientation in regard to the specimen removed. For this general purpose, a useful procedure is to saw the segment through in the sagittal plane, slightly to one side of the midline. This cut preserves the relationships of the intervertebral disks, vertebral bodies and spinal canal, as well as the other vertebral components. If one wishes tissue for microscopic study, one can further cut thin slices from one or the other of the halves (Figs. 12 and 13).

On the other hand, the vertebral segment may be cut in the frontal plane, the first cut starting just below the anterior longitudinal ligament. Serial slices about 3/16 in. in thickness may then be cut through the bodies until the spinal canal is reached (Figs. 14 and 15).

Then, again, one's interests may be such that one may wish to cut the vertebral segment in more than one longitudinal plane. This is done by first bisecting the specimen in the sagittal plane and then sawing one half serially in the sagittal plane and the other half serially in the frontal plane (Fig. 16).

If in a particular case one's primary interest is in the segment of the spinal cord, unroofing the vertebral canal gives one an excellent opportunity for examining the cord and the nerves coming off from it in relation to their setting. The unroofing is done by running the cut through the junction of the lamina with both pedicles. Injury to the cord can be averted if the cut is carried carefully through only two or three vertebrae at a time. After the cord has been unroofed, many of the vertebral foramina will be found still partially covered by a very thin fragment of lamina. By cutting the remains of the foramina away from the pedicles with shears, one then opens the foramina completely. Now one can examine the cord and the nerves as they course through the foramina, and the relationship of the intervertebral disks to the nerves as they emerge from the spinal canal (Fig. 17).

If one is especially interested in the costovertebral and costotransverse articulations, one can expose them in the three lower thoracic vertebrae by a single saw cut directed transversely through the rib and transverse process into the costovertebral joint and the body of the vertebra. In the remaining thoracic vertebrae, however, this cut will not include both facets of the costovertebral articulations. These can be demonstrated by sawing across the costovertebral articulation of both vertebral bodies in a plane almost perpendicular to the articular surface of the joint.

The intervertebral facet joints are exposed and their relationships preserved by a diagonally directed transverse cut. The single saw cut extends from the base of the spinous process above the facet joint through the joint, the pedicle, and the body of the vertebra below.

The intervertebral disks may be examined in relation to the vertebrae by serially slicing the specimen in either the sagittal or the frontal plane. However, is is often desirable to examine the intact disk, removed as completely as possible from the vertebral bodies. In order to do this with ease, one must first free the vertebral bodies by severing the pedicles in the frontal plane. The disks can then be shaved from the opposed surfaces of the bodies with a slicing knife.

SUMMARY

In this article we present a description of the procedure which we employ for removing a segment of the spinal column from the fourth or fifth thoracic through the fifth lumbar vertebra as part of our routine autopsy technique. We also give directions for reconstructing the column into a unit which will remain firm under subsequent handling of the body. In addition, there are briefly outlined various ways of gross sectioning of the column, directed toward the demonstration of particular pathologic alterations. Furthermore, in the paper is included information about tools and materials necessary for easy removal and reconstruction of a segment of the vertebral column. Special attention is given to the manner in which an ordinary small wood-cutting band saw has been adapted to the rigorous requirements involved in the proper cutting of bones and their overlying soft parts.

News and Comment

Dr. Anderson Goes to University of Miami.—W. A. D. Anderson, M.D., Professor of Pathology, Marquete University School of Medicine, Milwaukee, has accepted the position of Professor and Chairman of the Department, University of Miami School of Medicine, Coral Gables, Fla., and Director of the Pathology Laboratories, Jackson Memorial Hospital, Miami, Fla. Dr. Anderson will assume his new position on Aug. 1, 1953.

Books

An Introduction to Medical Science. By William Boyd, M.D. Third edition. Price, $3.50. Pp. 366, with 125 illustrations and one plate in color. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1952.

In his preface to this book Professor Boyd states that it "is a general introduction to the study of disease, an airplane view of that subject, its causes and bodily changes which accompany it. When one descends to earth again it is easier to understand the details of the country over which one has flown." This expresses very well the purposes of the book, which is obviously designed for nurses and laboratory technicians and as a general introduction to medicine for other interested persons. It presents the subject from the point of view of pathology. The first section deals with the general principles of pathology and the second section with the organs and their diseases. A third and final section discusses some of the practical applications of this knowledge, such as the principles of treatment and the collection of material for the laboratory.

Anyone familiar with Professor Boyd's other books would expect this one to be interestingly written, and it is. The material is well organized and clearly presented, and the illustrations are well chosen and well reproduced. The book is up-to-date and includes, for example, brief discussions of the interrelations of the endocrine glands and the part they play in stress and metabolism. One minor criticism which this reviewer would like to make is of the use of an archaic system of measurement. For instance, on page 233 the adrenals are described as being the size of a flat thimble.

On the whole, this is a well written book, which achieves its aim of being an elementary introduction to medicine through pathology. It should be useful for nurses, technicians, and others who have need for such a bird's-eye view of the natural history of disease.

Practical Blood Grouping Methods. By Robert L. Wall, M.D., Department of Research Medicine, The Ohio State University Hospital, Columbus, Ohio; formerly, Blood Research Section, Department of Biologic Products, Army Medical Department Research and Graduate School, Washington, D. C. Price, $5.00. Pp. 175, with numerous tables. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1952.

The title of this handy monograph restricts its scope to technical aspects of blood grouping. This highly specialized field has been developing so rapidly that it seems easier to write a new book on the subject than to prepare a new edition of a book published previously. That conclusion suggests itself to the reviewer as he watches new monographs on blood groups coming off the press in numbers which seem hardly justified by real needs.

This present volume, a monograph in the series of American Lectures in Hematology, covers the essentials of the subject in 12 brief chapters and 5 appendixes. They include a general introduction; a historical review; materials and reagents employed; some valuable data on methodology; more detailed discussion of the various blood group systems, with special emphasis on the Rh factor; a discussion of autoagglutinins and cold agglutinins, including antibodies associated with hemolytic anemia, and differential agglutination tests. The appendixes deal

with techniques of preparation of blood grouping, Rh typing and anti-human-globulin serums, and with complexities of the Rh nomenclature. Finally, there is a selected bibliography and a well-organized subject index.

This book is meant primarily for the laboratory worker and for the clinical pathologist. The method of presentation is clear and effective. It is regrettable that in addition to various minor and typographic errors a few fundamental defects have crept in, such as limitation of blood grouping to testing of unknown red cells. It is generally recognized that complete grouping requires, in addition, testing of the serum with known cells. Another example: Acacia and glue are listed as protein solutions.

On the whole, the book has many attractive features. The most important of them is emphasis on technical details and sources of error. It will be found useful in the laboratory.

Die Frühdiagnose des Uteruscarcinoms. By Prof. Dr. Med. Hans Limburg, Oberarzt an der Universitäts-Frauenklinik, Hamburg. Price, 19.50 German marks. Pp. 208, with 83 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1952.

This little book emphasizes the practical diagnosis of uterine cancer by means of biopsy and colposcopic, cytologic, and biochemical methods. About half of it is devoted to diagnosis by cytologic means, described in great detail. Yet the author emphasizes that the cytologic diagnosis of cancer should not be stressed too much and regards it as without value, and even dangerous, if practiced without the aid of colposcopic and histologic follow-up. The importance of recognizing carcinoma before it has started to infiltrate is continually stressed. In the chapter on biochemical methods as an aid in diagnosis of very early carcinoma, the triphenyltetrazolium chloride, glucuronidase, and phosphamidase tests are discussed, as are variations in the metabolism of benign and malignant lesions obtained in the Warburg apparatus. The book is well written, is easily understandable, and can well be recommended.

Atlas of Tumor Pathology. Section X, Fascicles 35 and 37, Tumors of the Central Nervous System. By James W. Kernohan, M.D., Section on Pathologic Anatomy, Mayo Clinic, Rochester, Minn.; Professor of Pathology, Graduate School, University of Minnesota, and George P. Sayre, M.D., Section on Pathologic Anatomy, Mayo Clinic, Rochester, Minn.; Instructor in Pathology, Graduate School, University of Minnesota. Price $0.90. Pp. 129, with 126 illustrations. Published by the Armed Forces Institute of Pathology, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, 2101 Constitution Ave., N.W., Washington 25, D. C., 1952.

In this very complicated segment of oncology the authors try to discuss "all neoplasms arising within the skull and spinal canal from the brain and spinal cord, meninges, nerve roots, blood vessels and extradural tissues." Pineal tumors are included, but those of the pituitary glands are not.

The first major subdivision is that of tumors of nervous tissue. The gliomas receive the greatest amount of space. As expounded in the present volume, the Mayo Clinic classification of astrocytomas into four grades, corresponding to the older terms of astrocytoma, astroblastoma, and glioblastoma multiforme, seems to accomplish but little in rendering the field more orderly. The other subdivisions of the glioma group include the ependymomas, oligodendrogliomas, medulloblastomas, neuroastrocytomas, and the very small group (not even illustrated) of "sub-ependymal glioma." Neurilemmoma and von Recklinghausen's disease are included under the first major category, correlative with gliomas. The other major headings are tumors of mesenchymal tissue (subdivided into blood vessel tumors, sarcomas, and lipomas), meningiomas, tumors of developmental defects (subdivided into paraphyseal cyst, chordoma, and dermoid and epidermoid tumors), and finally, tumors of the pineal body (originally planned as a separate fascicle).

The classification and choice of material leave much to be desired. "Tumors of the central nervous system" and "intracranial tumors" are not synonymous. The entire treatment and choice of subject matter seem to reflect principally the material available at the Mayo Clinic, rather than the subjects in their own right. Thus, lipomas of the brain are not mentioned, although

those of the cord are. Melanomas of the meninges are not mentioned. The inclusion of chordomas in a study of central nervous system tumors seems rather a strain. Pinealomas are discussed in very cursory fashion with only a single reference.

Many of the illustrations contribute but little, and photographs of gross material receive disproportionate emphasis. In general, the technical quality is good. The volume, however, appears too ambitious in conception but not entirely satisfactory in fulfillment. For the neophyte the fascicle is too brief, and for the skilled neuropathologist, not especially illuminating. It would have been preferable to have one fascicle devoted exclusively and thoroughly to the gliomas, another to meningiomas and mesenchymal tumors, and still another to miscellaneous intracranial neoplasms.

Atlas of Tumor Pathology: Section V, Fascicle 15, Tumors of the Parathyroid Glands. By Benjamin Castleman, M.D., Acting Chief, Department of Pathology and Bacteriology, Massachusetts General Hospital, and Assistant Professor of Pathology, Harvard Medical School. Price $0.65. Pp. 74, with 53 illustrations and 3 color plates. Published by the Armed Forces Institute of Pathology, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, 2101 Constitution Ave., N.W., Washington 25, D. C., 1952.

The term tumor, in this study of the parathyroid glands, is not restricted to the sense of neoplasia, but includes functional and nonfunctional enlargements. The author discusses enlargements of the gland under three headings: primary hyperparathyroidism, which includes adenoma, carcinoma, and primary hyperplasia and hypertrophy; a secondary hyperparathyroidism, and nonfunctional enlargements, including cysts, carcinomas, and oxyphile "adenomas." There is a very brief discussion of the normal gland, and, in addition to the discussion and illustrations of the parathyroid glands, there are some fine photographs of renal calcinosis and osteitis fibrosa cystica.

The illustrations are excellent throughout, but the text is uncomfortably brief. The problem of how much text an atlas should include is indeed moot, but to the reviewer the present volume misses a skillful balance. The references also are rather scanty. Nevertheless, the fascicle is an important volume, which pathologists and teachers will find useful.

Nutrition in the Practice of Medicine, with Comments on Nutrition, Disease and Geography. Proceedings of the Nutrition Symposium Held at the University of California, School of Medicine, San Francisco, October 30, 1951: Nutrition Symposium Series Number 4. By J. Arnold Bargen, Paul R. Cannon, John B. Condliffe, Perry J. Culver, Robert M. Kark, Heinrich Necheles, and Frederick J. Stare. Price, $1.50. Pp. 163. The National Vitamin Foundation, Inc., 150 Broadway, New York 38, 1952.

Heredity in Uterine Cancer. By Douglas P. Murphy, M.D., F.A.C.S., Assistant Professor of Obstetrics and Gynecology and Research Associate, Gynecean Hospital, Institute of Gynecologic Research, University of Pennsylvania. Price, $2.50. Pp. 128, with 51 tables. Published for the Commonwealth Fund by Harvard University Press, Cambridge 38, Mass., 1952.

This work is based on a detailed statistical study of the incidence of cancer in the families of 201 women who had carcinoma of the uterine cervix and of 215 women in the same age group who did not have cancer of the cervix. Extensive questionnaires were used, and many sources of information in addition to family interviews were probed in order to learn causes of death, names of physicians, etc.

The cancer patients were found to be of a somewhat lower economic and educational level than the controls, to have married earlier, and to exhibit more frequent interruption of marriage by widowhood, divorce, and separation. Curiously, no data regarding the number of pregnancies of the subjects are reported, although it is noted that the cancer patients had a higher incidence of abortions.

The family studies indicate that heredity affects the frequency with which cancer occurs in the uterus, the incidence in mothers and aunts of the cancer subjects being twice that in the control families. Cancer in other sites occurred with equal frequency in the two groups of families, however, indicating that the predisposing factor is in some measure specific.

The monograph includes a detailed exposition of the methods used in amassing data of this type and is interesting from this standpoint. The significant information resulting from the study, however, comprises a very small fraction of the text.

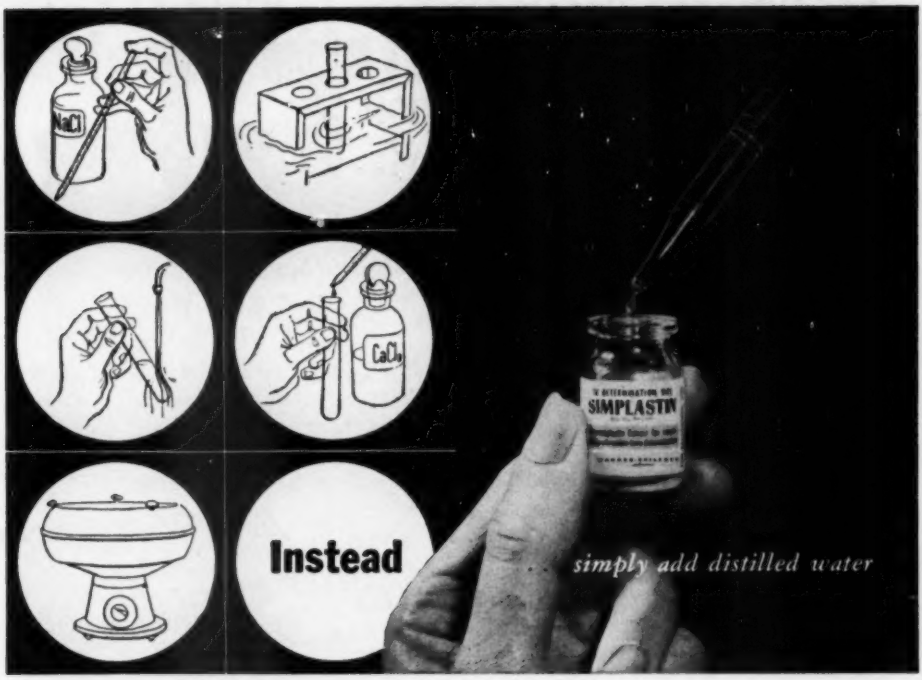
Basic Medical Physiology. By W. B. Youmans, Ph.D., M.D., professor of physiology, University of Wisconsin, Madison, Wis. Price, $7.50. Pp. 436, with 117 illustrations. Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1952.

To summarize the present basic knowledge of physiology in little more than 400 pages is not an easy task. The author has succeeded in this only in part. Equilibrium between various sections of the book has not been completely achieved. For the sake of simplicity and brevity many important subjects are either dismissed in a few lines or completely omitted. The style, however, is lucid, and the numerous diagrams are clear and, for the most part, well chosen. This book cannot be recommended to the medical profession, although it might find its place in the teaching of premedical college students, nurses, and medical technicians.

Die Krankheiten der Nasennebenhöhlen, der Ohren und des Halses im Röntgenbild (45. Ergänzungsband der "Fortschritte auf dem Gebiete der Röntgenstrahlen vereinigt mit Röntgenpraxis"). Second edition. By Prof. Dr. Richard Mittermaier, Direktor der Universitäts Hals-Nasen-Ohren Klinik, Marburg/Lahn. Second edition. Price, 66 German marks. Pp. 232, with 483 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1952.

This is a well-written monograph by an experienced clinician. In the introduction the author includes a short résumé of the topographic anatomy of the sinuses and of the temporal bone. He states that roentgen film should serve only as a supplement to the clinical findings and that the diagnosis should not depend upon the x-ray film alone. The major portion of the book deals with roentgenographic considerations of the diseases of the paranasal sinuses and the temporal bone. The chapter dealing with sinus disease is most comprehensive. Six principal positions are described in detail and supported by well-done drawings showing the technique and the important anatomical structures and landmarks. The positions are mainly designated anatomically, for instance, occipitonasal instead of Water's position. The author prefers the vertical arrangement with the patient in sitting position, the head being adjusted to the vertical film. This method makes possible the detection of fluid levels in the sinuses. Short clinical notes are added to typical films demonstrating pathology of acute and chronic sinusitis. Various exposures and directions are frequently necessary for exact localization of a diseased ethmoid sinus. In the second chapter the author discusses pathological changes of the temporal bone as seen in the x-ray film. He prefers the lateral view of Schuller and the posterior-anterior position of Stenver to delineate the osseous changes in acute and chronic mastoiditis. For tumors in the pars petrosa, Mayer's position and technique are mentioned but not utilized in the interpretation. Ronentgenography of the pharynx and of the larynx is briefly presented. The author suggests the employment of tomography and advocates the method suggested by Rethi and Waldapfel, which requires local anesthesia to introduce the film into the hypopharynx.

There are 483 excellently reproduced films, all in positive printing, this being the standard in German publications. There are no references included in the text. Although no new roentgenographic methods or interpretations are added, the book should be of value to students and clinicians who prefer to read the films of their patients themselves.



simpler prothrombin time determinations

Accurate prothrombin time determinations are required in effective anticoagulant therapy. Simplastin insures greater accuracy, reduces the time factor from 45 to 5 minutes and requires only the addition of distilled water—in contrast to older, complicated methods. Sources of variability and dependence on technical dexterity are eliminated.

With Simplastin "the operator need only add a stated amount of distilled water and the material is ready for use."[1] Thus, "many costly and cumbersome steps are eliminated in preparation."[2] Results are readily reproducible.

Simplastin gives "considerable assurance and safety" to the clinician "who has hesitated to employ Dicumarol therapy because of the difficulty encountered in laboratory control."[1]

Simplastin makes available prothrombin time tests in which errors due to the human element in "extracting, centrifugation or filtering are entirely eliminated."[1]

For the laboratory, **Simplastin "simplifies the procedure . . ."[3]**

For the clinician, **Simplastin "eliminates sources of serious error"[3] in prothrombin time determinations.**

Available: 6-Determination Size and 20-Determination Size, both in boxes of 10 vials.

A trial supply and literature on request.

Bibliography: 1. Shapiro, S., et al.: Am. Heart J. 40:766 (Nov.) 1950. 2. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. 222:207 (Aug.) 1951. 3. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey 48:52 (Feb.) 1951.

Simplastin®

WARNER-CHILCOTT *Laboratories,* NEW YORK

clean all this glassware

IN 1 HOUR... WITHOUT BREAKAGE!

...WITH THE EASY-TO-USE

FISHER

LABORATORY GLASSWARE WASHER

The above 917-piece load . . . including 500 Kahn tubes, 32 Petri dish components, 11 Erlenmeyer flasks, 8 beakers, and 366 serological pipettes . . . was delivered sparkling clean by the Fisher Laboratory Glassware Washer *one hour after the soiled glassware was placed in the washer cabinet.*

Not a piece of glassware was broken . . . and the operator was free for much of the hour to handle other duties.

This case is typical of the way the Fisher Washer meets every glassware cleaning need for your industrial, research, hospital, or clinical laboratory.

Special baskets are available to hold all types of laboratory glassware. You simply fill the proper baskets with the items to be cleaned . . . attach to revolving drum inside cabinet . . . add detergent . . . and turn washer on.

The machine washes, rinses, steams, and dries; removes salt deposits, blood clots, agar, rings, precipitates and wax pencil marks. It also solves a critical personnel problem by replacing an unpleasant, full-time chore with a simple, once-a-day mechanical operation.

Two models are available: (1) Steam-Heated and (2) Electrically-Heated.

Complete stocks of laboratory instruments, apparatus, reagent chemicals, furniture, and supplies at PITTSBURGH, NEW YORK, ST. LOUIS, WASHINGTON, MONTREAL and TORONTO. For more information write: Fisher Scientific Co., 717 Forbes St., Pittsburgh 19, Pennsylvania.

FISHER SCIENTIFIC

America's Largest Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals

Paragon Tray Drawer Cabinet

Compact

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¼

Low Cost

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES
2 x 2" SLIDES
LANTERN SLIDES
(up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET YOU PURCHASE FILING SPACE ONLY NO WASTE SPACE-EVERY INCH USED

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimnsions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3⅞
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.
Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.

the new *Lab-aid* way to store and view color transparencies

Assembly of Lab-aid Transparency Tray Cabinets, with the illuminator cabinet drawer pulled out. Use the table base, or stack them with your present Lab-aid Files.

You know how efficiently Technicon Lab-aid cabinets bring order to the filing of microslides, paraffin blocks and associated materials in the pathologic laboratory.

Now, in this new addition to the Lab-aid Filing System, you can enjoy the same benefits in the filing and viewing of color transparencies and lantern slides. The new Lab-aid transparency file has 40 smooth-sliding flat trays, each holding 16 standard 2" mounts, or 5 3¼" x 4¼" lantern slides in full view. They're easy to insert . . . you just drop them flat in the grooved frames. Easy to remove, too, by popping them out from underneath.

The special cabinet with its pull-out illuminator drawer lets you view a whole tray of transparencies at a glance, without removing any of them. Sitting or standing, you can inspect hundreds of slides under ideal viewing conditions without stirring from your place. And the disappearing shelf is a mighty handy place to lay slides down temporarily while you're working with them.

Both tray and illuminator cabinets are of the same dimensions and finish as all the other units in the Technicon Laboratory Filing System, and can be stacked interchangeably with them. If you would like more information about these or other units of the system, we'll be glad to send details.

The disappearing shelf is convenient as a temporary resting place for slides while working with them.

Inspect all your slides without removing any of them, or even stirring from your place.

Other units in the Lab-aid line

1" MICROSLIDE DRAWER FILES
2" SLIDE OR TRANSPARENCY DRAWER FILE
4" SLIDE OR INDEX DRAWER FILES
MICROSLIDE FLAT-FILING TRAY CABINET
PARAFFIN BLOCK FILE

technicon *Lab-aid* filing system

THE TECHNICON COMPANY
215 East 149th St., New York 51, N. Y.